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United States Patent [19]

Zhu

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[54] **SYRINGE CONTAINING DRUG TO BE
INJECTED**

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[21] Appl. No.: **565,283**

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[30] **Foreign Application Priority Data**

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[51] Int. Cl.⁶ **A61M 31/00**

[52] U.S. Cl. **604/82; 604/85; 604/89;
604/202; 604/236; 604/246**

[58] **Field of Search** **604/83, 85, 87,
604/89-91, 131, 140-1, 143, 146-9, 181-4,
187, 197, 200-6, 218, 222, 226-9, 231,
233-4, 236-7, 249, 256; 137/845**

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Chestnut*

[57] **ABSTRACT**

A syringe containing drug to be injected, the syringe includes an injection tube, a needle mounted at the front end of the injection tube, a rubber sealing jacket stopper jacketing on the peak of the needle and an injection tube core movably and hermetically put inside the injection tube, which is characterized in that the injection tube core consists of an ampule of cylindrical bottle shape and a piston, the back end of the ampule is closed and at its front end there are a flange and a neck portion, the central through hole of the flange and the neck portion is interpenetrated with the inner cavity of the bottle-shaped injection tube core; the outside top of the piston appears as a cone, and its outside lower part is a cylinder matching the inner wall of the injection tube, while the inside top of the piston appears as an inner cone, and the form of its inside lower part matches the shapes of the flange and the neck portion located at the front end of the ampule; at the cone-shaped top of the piston there is a slit linking up the inside and the outside of the top and inclined to the axis of the piston, so that a one-way valve which could be opened outwardly is formed at the cone-shaped top.

3 Claims, 2 Drawing Sheets

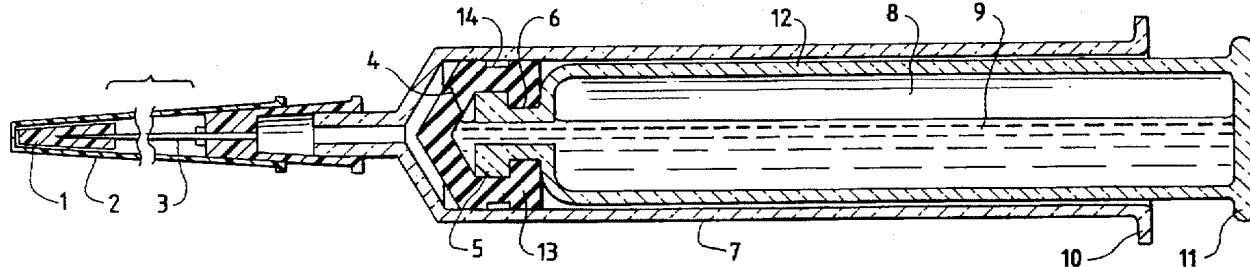


FIG. 1

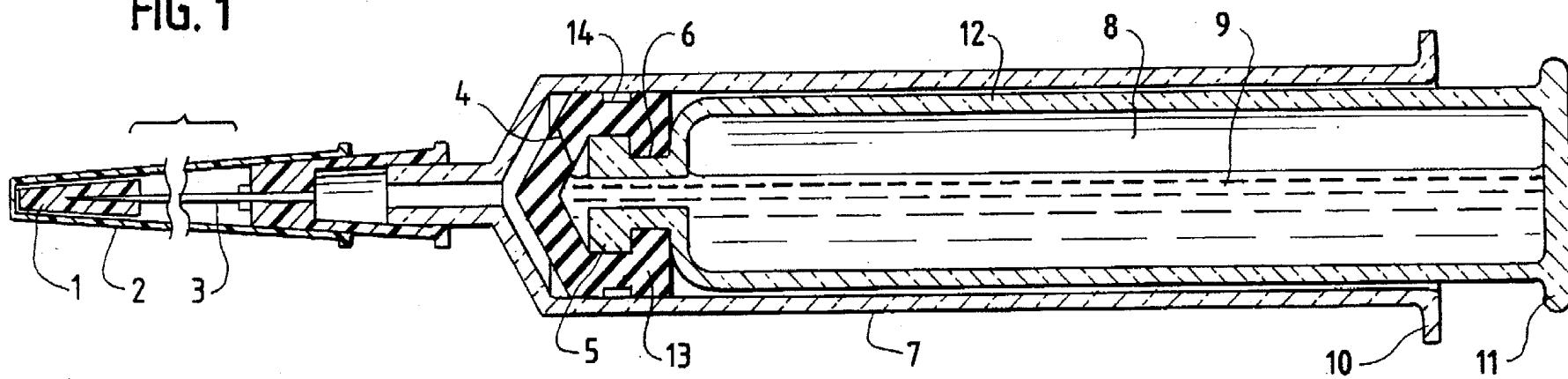


FIG. 2

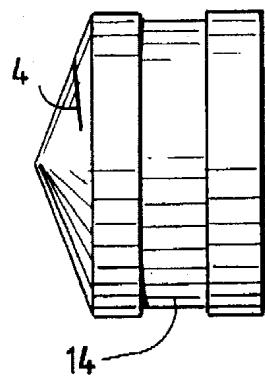


FIG. 3

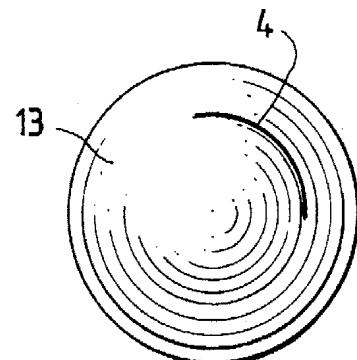


FIG. 4

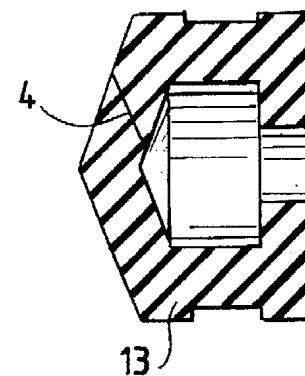
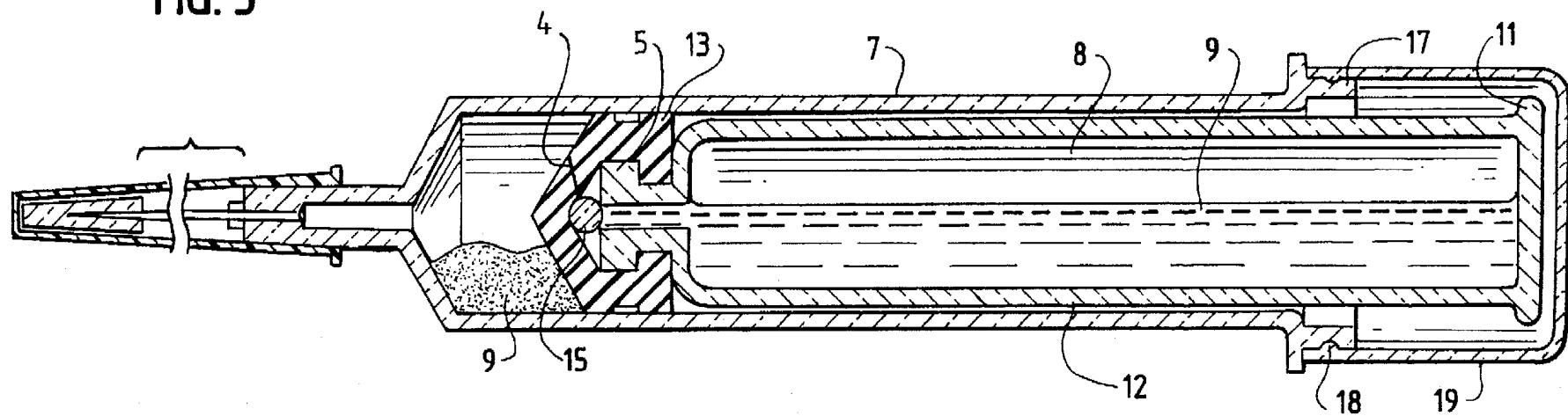
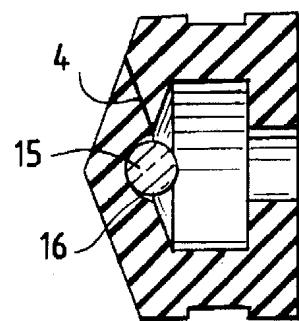
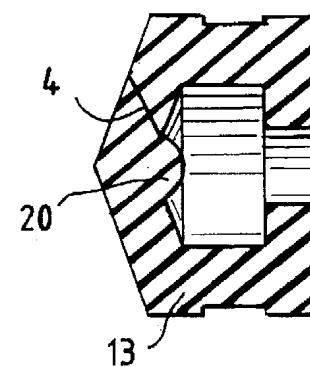


FIG. 5**FIG. 6****FIG. 7**

SYRINGE CONTAINING DRUG TO BE INJECTED

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a medical syringe, especially to a disposable syringe containing drug to be injected, particularly to a syringe, which has been provided with two functions for injecting and containing drug, i.e. which is both a bottle for storing and transporting drug, namely so-called ampule, and a drug syringe for injecting drug into the body in need of injection.

2. Prior Art

The medical and disposable aseptic syringe in present clinical usage includes an injection tube and an injection tube core, one end of the injection tube is open and at its other end there is a nipple for mounting needle, the injection tube core is movable and hermetically put in the injection tube. The bottle containing drug, i.e. ampule, is used for storing and transporting drug. When a user needs injection, it is necessary to cut and open the ampule at first, then to suck the drug from the ampule into injection tube through needle in preparation for injection. The operation for manipulating such kind of syringe is quite complicated, its working efficiency is low, particularly, during cutting and opening the ampule, the broken glass might be sucked into the injection tube and further injected into human body, so that a very serious consequence could result.

Accordingly, a kind of disposable syringe containing drug has been proposed, the injection tube of the syringe has a bypass linking up axially its front and back parts, both the inlet and the outlet of the by path are located on the inner surface of the injection tube, hence, the inner cavity of the injection tube is interpenetrated with the by path. The injection tube core consists of a rod, on which several pistons, for example 2 pistons, are provided at intervals, the axial interval between two pistons matches one between the inlet and the outlet of the bypass. When the injection tube core is movable and hermetically put in the injection tube, these pistons, for example two pistons, cover hermetically the inlet and the outlet respectively. The drug can be stored in the front part of the cavity of the injection tube, and the thinner is stored between two pistons. During application, when the injection tube core is being pulled out, its pistons don't seal the outlet and the inlet of the bypass any longer, at that time, in the front part of the injection tube vacuum results, because the needle is stopped by a rubber sealing stopper, and the thinner contained between the pistons inside the injection tube flows into the front part of the injection tube and mixes with the drug therein, then, remove the sealing stopper from the needle, push forward the injection tube core, after its front end has passed beyond the outlet of the bypass, the drug liquid can be forced for injection.

However, for the prior art disposable syringe integrating the syringe and the ampule into a whole, the structure is rather complicated, particularly, the stored drug is possible to be polluted, because the drug is isolated from outside only by the sealing between the pistons and the inner wall of the injection tube, hence the sealing is not very successful due to the movement of the pistons.

Therefore, it is an object of the present invention to provide a kind of disposable syringe integrating the syringe and the ampule into a whole, for which the structure is simple, and the stored drug can be sealed successfully and preserved not to be polluted and deteriorative for a long time.

SUMMARY OF THE INVENTION

With the above-mentioned aim, the present invention provides a syringe containing drug to be injected, the syringe includes an injection tube, a needle mounted at the front end of the injection tube, a rubber sealing jacket stopper jacketing on the peak of the needle and an injection tube core movably and hermetically put inside the injection tube, which is characterized in that said injection tube core consists of an ampule of cylindrical bottle shape and a piston, for said ampule, its back end is closed, and at its front end there are a flange and a neck portion, the central through hole of the flange and the neck portion is interpenetrated with the inner cavity of the bottle-shaped injection tube core; the outside top of said piston appears as a cone, and its outside lower part is a cylinder matching the inner wall of the injection tube, while the inside top of said piston appears as an inside cone, and the form of its inside lower part matches the shapes of the flange and the neck portion located at the front end of said ampule; at the cone-shaped top of said piston there is a slit linking up the inside and the outside of the top and inclined to the axis of the piston, so that a one-way valve which could be opened outwardly is formed at the cone-shaped top.

Said slit on the piston could be a partial arc, occupying an arc length of $\pi/4$ to $\pi/2$ radian.

Alternatively, the inside cone-shaped peak of the inside top of said piston is a concave sphere, a glass ball is located between said concave spherical peak and the outlet of the central through hole in the flange.

Alternatively, the inside cone-shaped peak of the inside top of said piston is a partial sphere which is convex inwardly.

On the cylindrical lower part of said piston there might be some ring grooves at intervals.

At the back end of said injection tube there are a protruding edge favorable for holding and pulling it with fingers, and an extending portion on which a cap is mounted to cover and seal the open end of the injection tube.

In the syringe containing drug to be injected according to the present invention, the liquid to be injected is contained in the injection tube core, when the injection tube core is pulled backwardly, at the front end of the inner cavity of the injection tube vacuum results, and the liquid in the injection tube core flows into the inner cavity of the injection tube through the one-way valve, which is formed by the inclined slit located on the top of the piston mounted at the front end of the injection tube core and could be opened outwardly. Remove the rubber sealing jacket stopper from the needle, push forward the injection tube core, then the liquid in the inner cavity of the injection tube sprays out under the press of the piston.

For such kind of syringe, not only the structure is simple, but also the drug sealed therein can be preserved successfully and the manipulation is convenient too.

BRIEF DESCRIPTION OF DRAWINGS

Further objects and advantages of the present invention will appear from the following description taken together with the accompanying diagrammatical drawings, where

FIG. 1 is an assembly sectional view of the first embodiment of the syringe containing drug to be injected in accordance with the invention;

FIGS. 2 and 3 are respectively a front view and a top one of the piston in the syringe illustrated in FIG. 1;

FIG. 4 is a sectional view of the piston in the syringe illustrated in FIG. 1;

FIG. 5 is an assembly sectional view of the second embodiment of the syringe in accordance with the invention;

FIG. 6 is a sectional view of the piston in the syringe illustrated in FIG. 5, where the sealed glass ball is shown;

FIG. 7 is a sectional view of the piston of the third embodiment in accordance with the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As shown in FIG. 1, in the first embodiment of the syringe in accordance with the invention, the syringe includes an injection tube 7, a needle 3 mounted at the front end of the injection tube 7, a rubber sealing jacket stopper 1 jacketing on the peak of the needle 3 and an injection tube core 8 movably and hermetically put inside the injection tube. On the needle 3 and the jacket stopper 1, a protecting case 2 is jacketed in order to protect the needle 3 against damage during transportation, the back end of the injection tube 7 is open, through which the injection tube core 8 can be inserted, and possesses a protruding edge 10 favorable for holding it with fingers. The injection tube core 8 includes an ampule 12 of cylindrical bottle shape and a piston 13, the back end of the ampule 12 is closed and possesses a flange plate 11 favorable for holding it with fingers, and at its front end there are a flange 5 and a neck portion 6, the central through hole inside the flange 5 and the neck portion 6 is interpenetrated with the inner cavity of the injection tube core 8. The piston 13 is jacketed on said flange 5 and neck portion 6, the outside top of the piston 13 appears as a cone, and its outside lower part is a cylinder matching the inner wall of the injection tube 7, on the lower part of the cylinder there might be one or more ring grooves 14 at axial intervals to form labyrinth sealing, so that the sealing will have better effect during sliding of the piston 13 (refer to FIGS. 2, 3 and 4). The inside top of the piston 13 appears as an inner cone and the form of its inside lower part matches the shapes of the flange 5 and the neck portion 6 located at the front end of the ampule 12. At the cone-shaped top of the piston 13 there is a slit 4 inclined to the axis of the piston 13, the slit 4 is a partial arc occupying an arc length of $\pi/4$ to $\pi/2$ radian in the circumferential direction, so that a one-way valve which could be opened outwardly is formed at the cone-shaped top of the piston 13. The drug liquid to be injected is preserved inside the ampule 12.

During application, at first pull the ampule 12 backwardly, because the needle 3 is stopped by the jacket stopper 1, at the front end of the injection tube 7, i.e. in front of the piston 13, vacuum results when the piston 13 slides on the inner wall of the injection tube 7, and the drug liquid 9 contained inside the ampule 12 flows into the inner cavity of the injection tube 7 through the one-way valve formed by the slit 4 on the piston 13. Then remove the protecting case 2 and jacket stopper 1, push forward the ampule 12, the drug liquid sprays out from the needle 3 under the press of the piston 13.

FIG. 5 shows the syringe of the second embodiment in accordance with the invention. This embodiment is basically the same as the first one, the only difference lies in that there is a spherical concavity at the inside top of the piston 13, i.e. the inside top is a concave sphere (refer to FIG. 6), and a glass ball 15 is located between the concavity 16 and the flange 5 at the front end of the injection tube core 8. The glass ball 15 can prevent drug from deterioration due to its long term contacting with the rubber piston. On the protrud-

ing edge 10 of the injection tube 7 there is a axially extending portion 17, on which a push-button structure 18 is provided, and a cap 19 is mounted on the extending portion 17 using its push-button structure. The drug liquid 9 is contained in the ampule 12, and the drug powder 9' in the inner cavity at the front end of the injection tube 7.

FIG. 7 is a sectional view of the piston 13 of the third embodiment in accordance with the invention. This embodiment is basically the same as the first one, the only difference lies in that at the inside top of the piston 13 there is a partially spherical portion 20, which is convex inwardly. When the piston 13 is mounted on the flange 5 and the neck portion 6 at the front end of the ampule 12 of the injection tube core 8, the partially spherical portion 20 covers hermetically the through hole in the flange 5, so that seals the ampule 12. When the ampule 12 is pulled backwardly, the partially spherical portion 20 doesn't cover the through hole in the flange 5 any longer because the one-way valve formed by the slit 4 turns to open outwardly, drug liquid flows from the inside of the ampule 12 into the injection tube 7 and is ready for injection.

What is claimed is:

1. An improved syringe containing drug to be injected, the improvement comprising an injection tube having a front end and an open back end, a needle mounted at the front end of the injection tube, a rubber sealing jacket stopper jacketing the peak of the needle and an injection tube core movably and hermetically put inside the injection tube, said injection tube core consisting of an ampule of cylindrical bottle shape, an inner cavity, and a piston, the back end of said ampule is closed, toward the front end of said ampule there is a neck portion, and immediately forward of said neck portion, the front end of said ampule terminates at a flange, said neck portion and said flange having a central through hole therein;

the central through hole of said flange and said neck portion is coextensive with the inner cavity of said bottle-shaped injection tube core;

said piston has a forward outside end which appears as a cone, a rear outside part which is a cylinder matching the inner wall of said injection tube, a forward inside end which appears as an inner cone, and a rear inside part having a form which matches the shapes of said flange and said neck portion located at the front end of said ampule;

at said cone-shaped forward end of said piston there is a slit linking up the forward outside end and the forward inside end and inclined to a longitudinal axis of said piston, so that a one-way valve which could be opened outwardly is formed at said cone-shaped forward end, said slit on said piston being a partial arc, occupying an arc length of $\pi/4$ to $\pi/2$ radian.

2. A syringe as claimed in claim 1, wherein said inside cone-shaped peak of said forward inside end of said piston includes a concave hemi-spherical portion therein, a glass ball is located between the apex of said concave hemi-spherical portion and the outlet of said central through hole in said flange.

3. A syringe as claimed in claim 1, wherein said inside cone-shaped peak of said forward inside end of said piston includes a rearwardly projecting convex hemi-spherical portion thereon.



US008048297B2

(12) **United States Patent**
Leach et al.(10) **Patent No.:** US 8,048,297 B2
(45) **Date of Patent:** Nov. 1, 2011(54) **METHOD AND APPARATUS FOR COLLECTING BIOLOGICAL MATERIALS**(75) Inventors: **Michael D. Leach**, Warsaw, IN (US); **James M. McKale**, Syracuse, IN (US)(73) Assignee: **Biomet Biologics, LLC**, Warsaw, IN (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1216 days.

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(22) Filed: May 3, 2007

(65) **Prior Publication Data**

US 2007/0208321 A1 Sep. 6, 2007

Related U.S. Application Data

(63) Continuation-in-part of application No. 11/210,005, filed on Aug. 23, 2005, now Pat. No. 7,771,590.

(60) Provisional application No. 60/900,758, filed on Feb. 9, 2007.

(51) **Int. Cl.**
B04B 5/02 (2006.01)(52) **U.S. Cl.** 210/109; 210/782; 210/789; 210/515; 210/516; 210/518; 494/16; 494/19(58) **Field of Classification Search** 210/515, 210/109, 518; 494/16, 19

See application file for complete search history.

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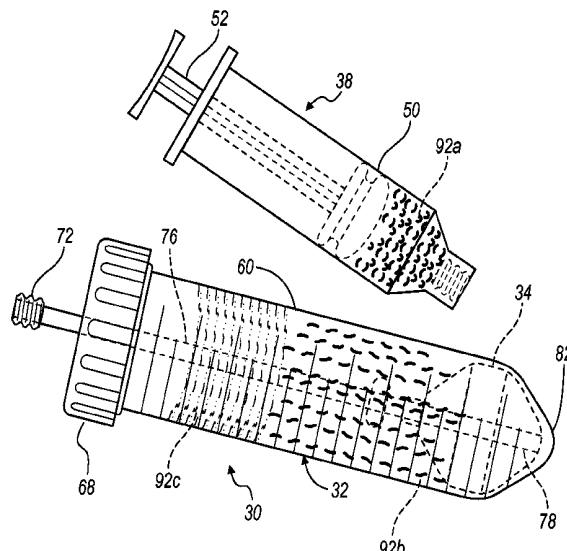
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Primary Examiner — Tony G Soohoo*Assistant Examiner* — David C Mellon(74) *Attorney, Agent, or Firm* — Harness, Dickey(57) **ABSTRACT**

A method and apparatus can separate and concentrate a selected component from a multi-component material. The multi-component material may include a whole sample such as adipose tissue, whole blood, or the like. The apparatus generally includes a moveable piston positioned within a separation container and a withdrawal tube that is operable to interact with a distal end of the collection container past the piston. Material can be withdrawn through the withdrawal tube.

17 Claims, 9 Drawing Sheets



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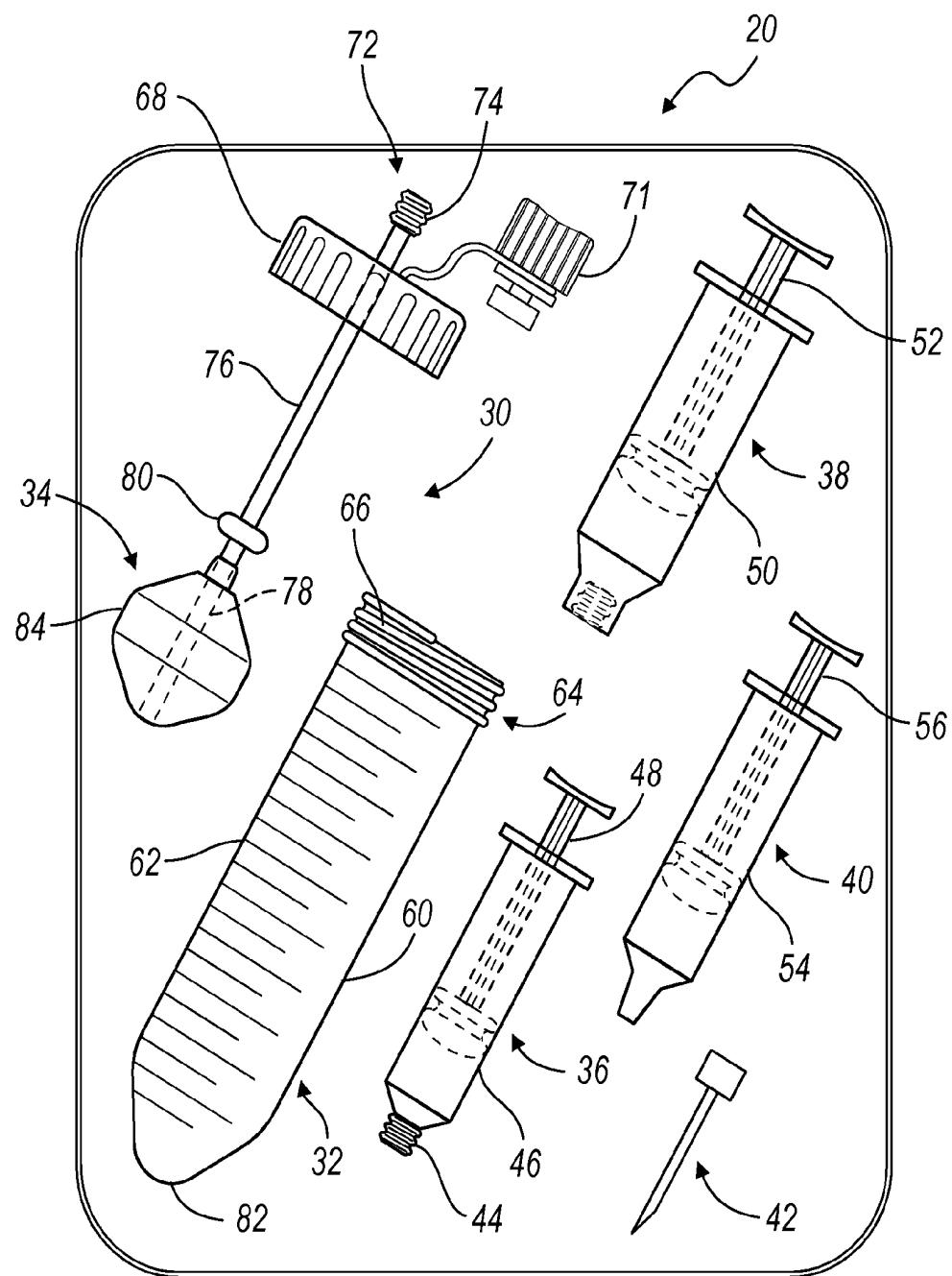
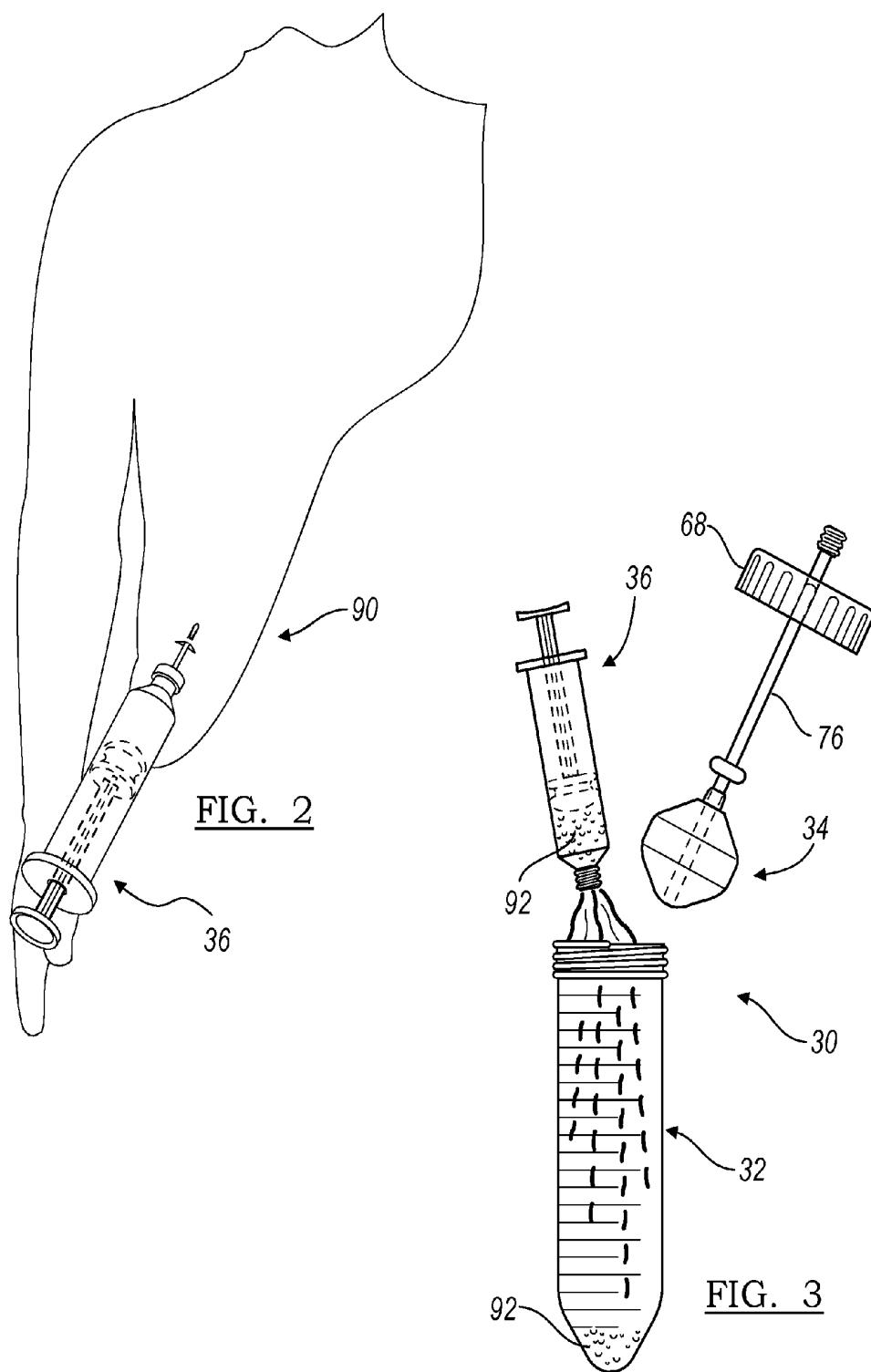
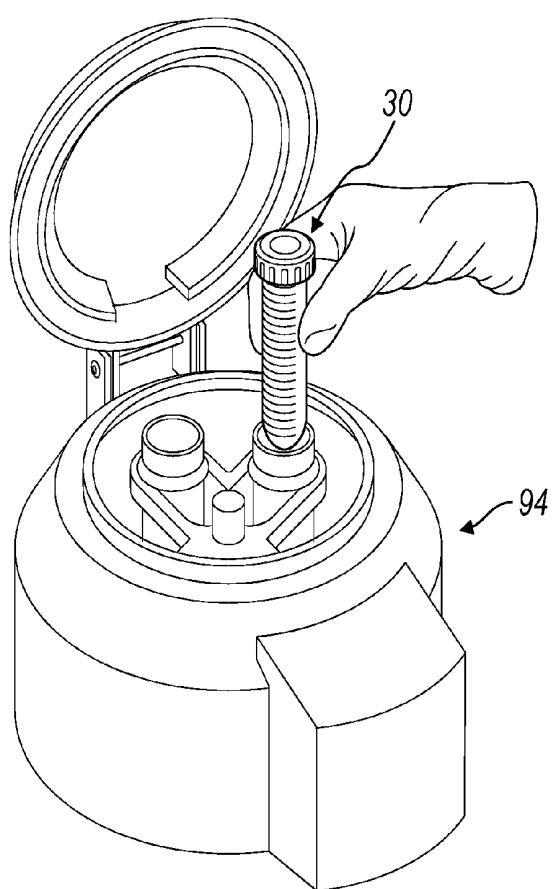
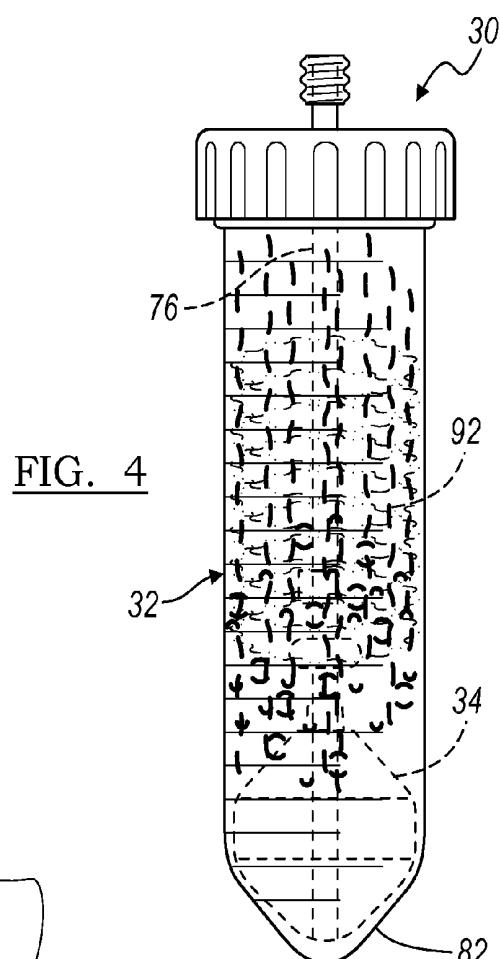


FIG. 1





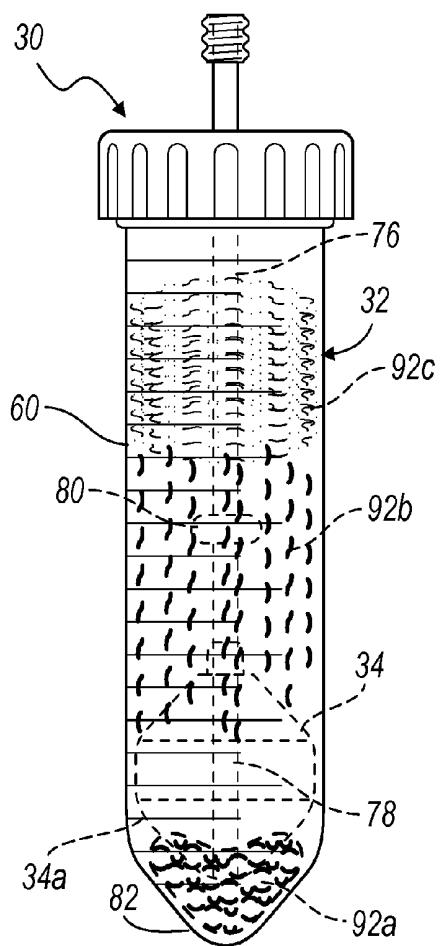


FIG. 6

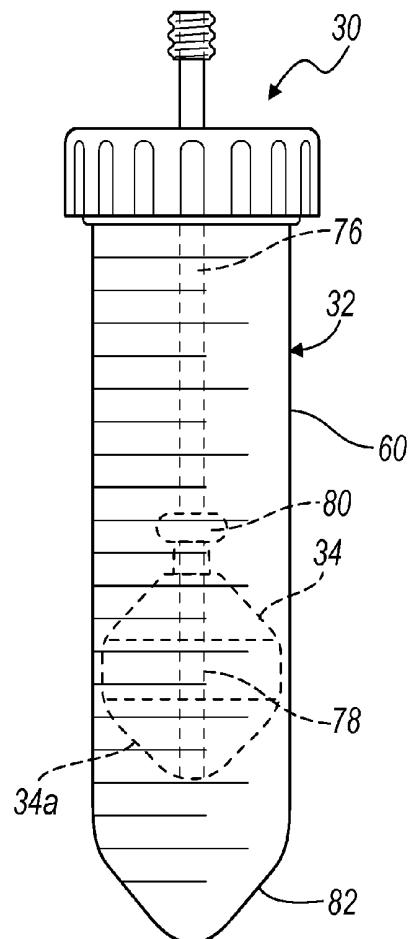


FIG. 6A

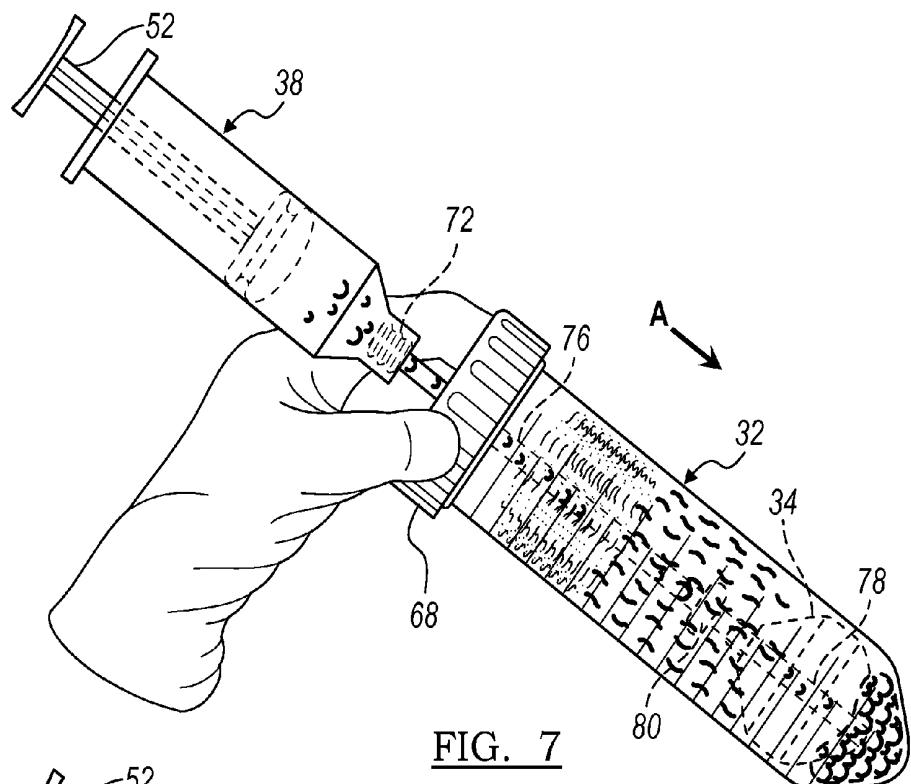


FIG. 7

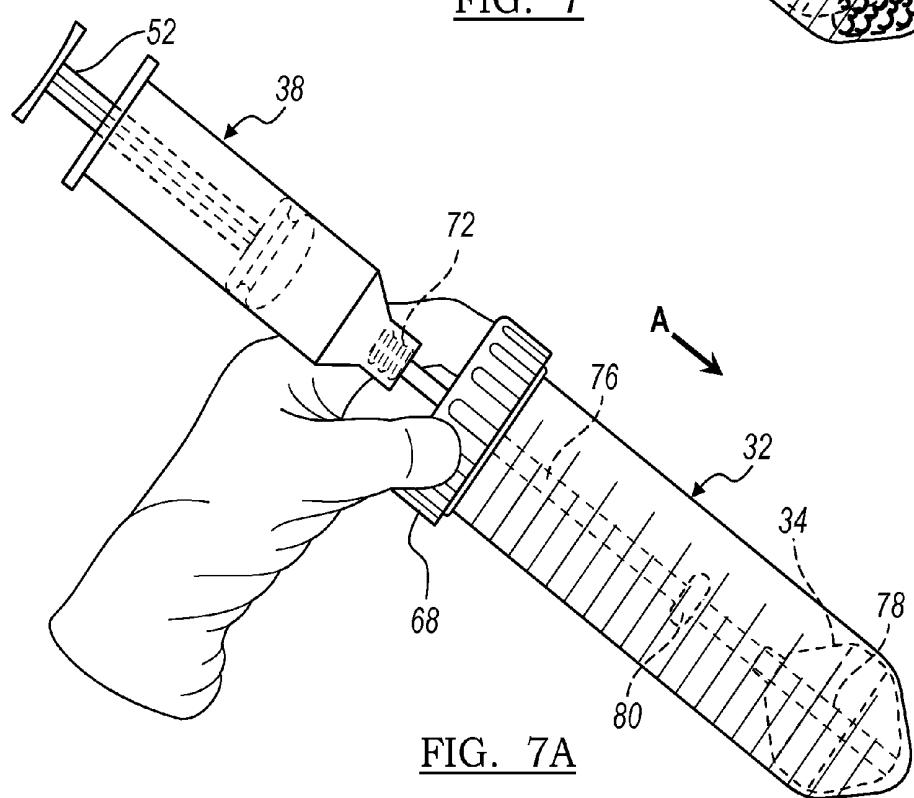


FIG. 7A

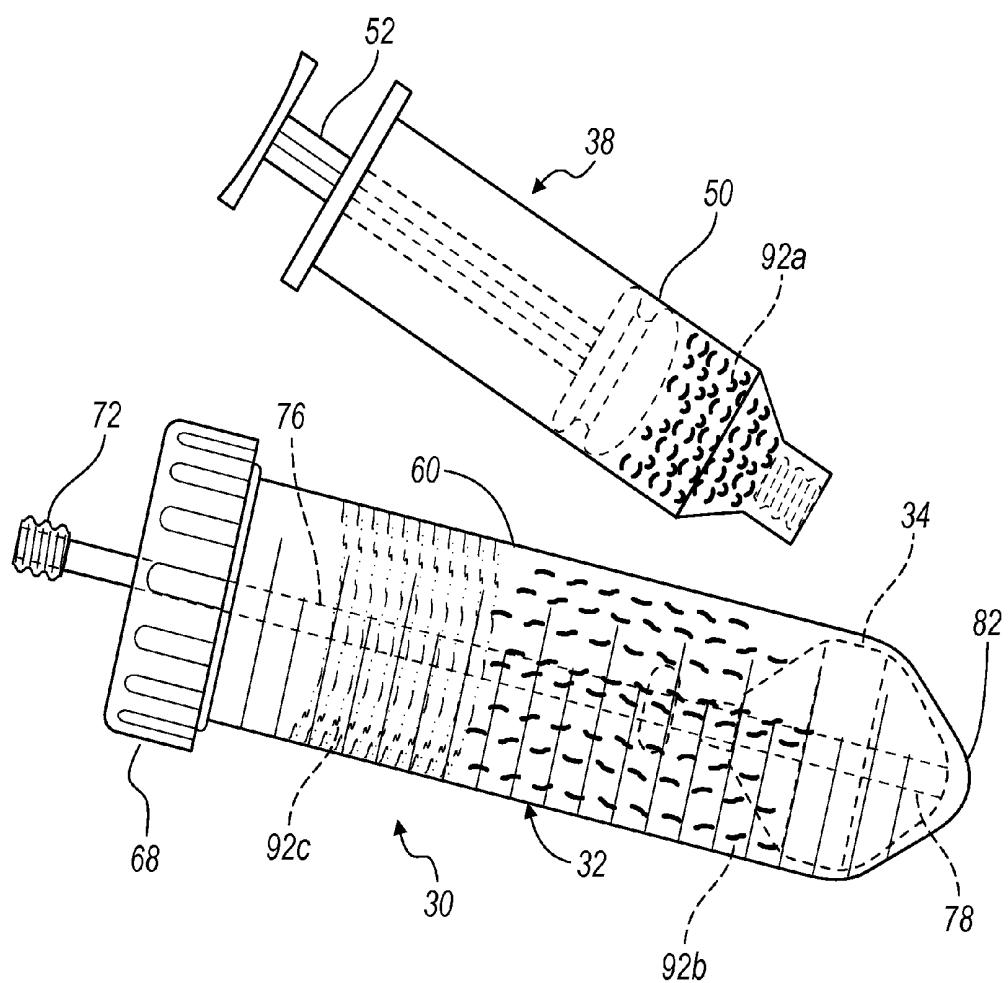
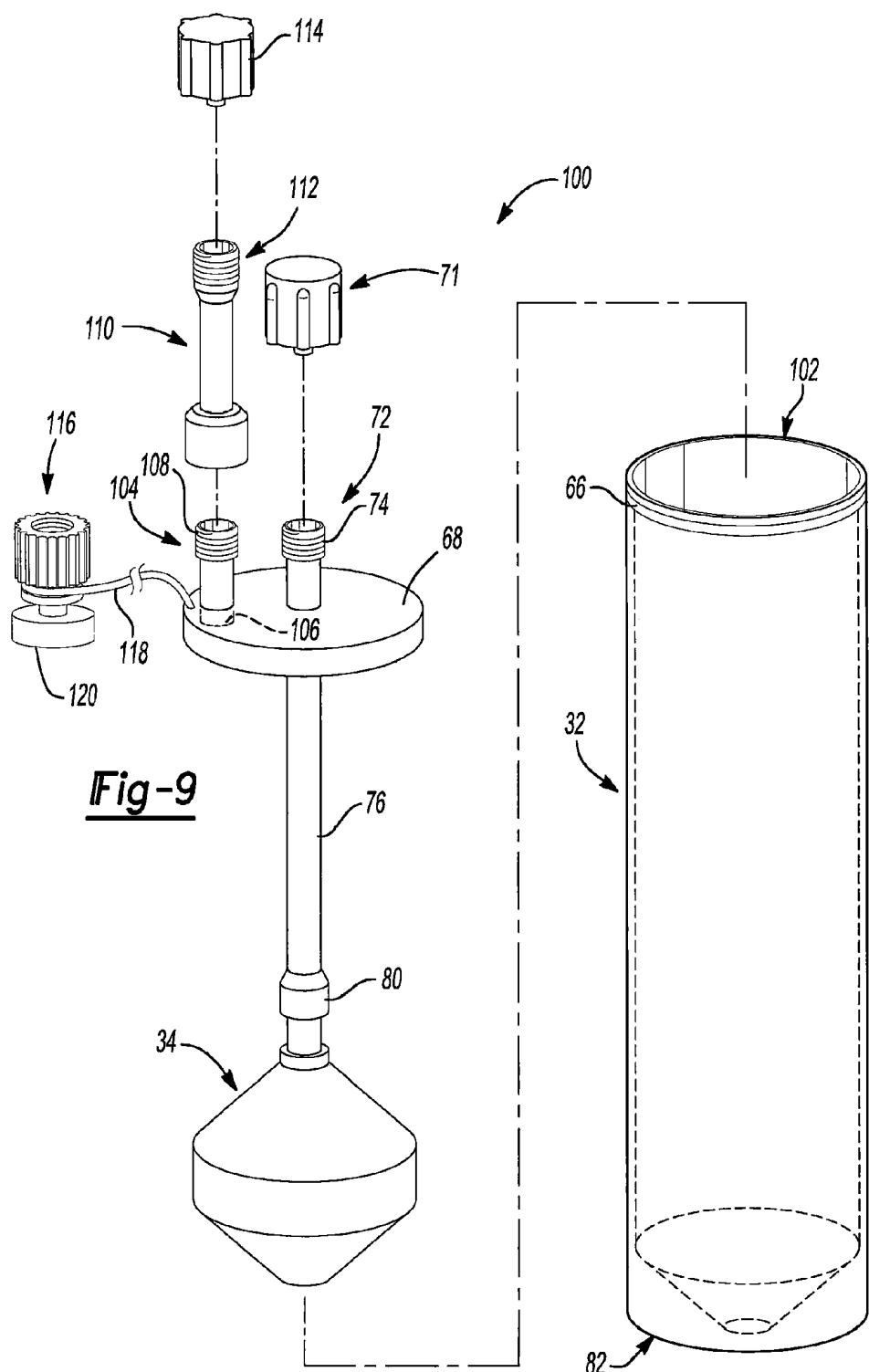
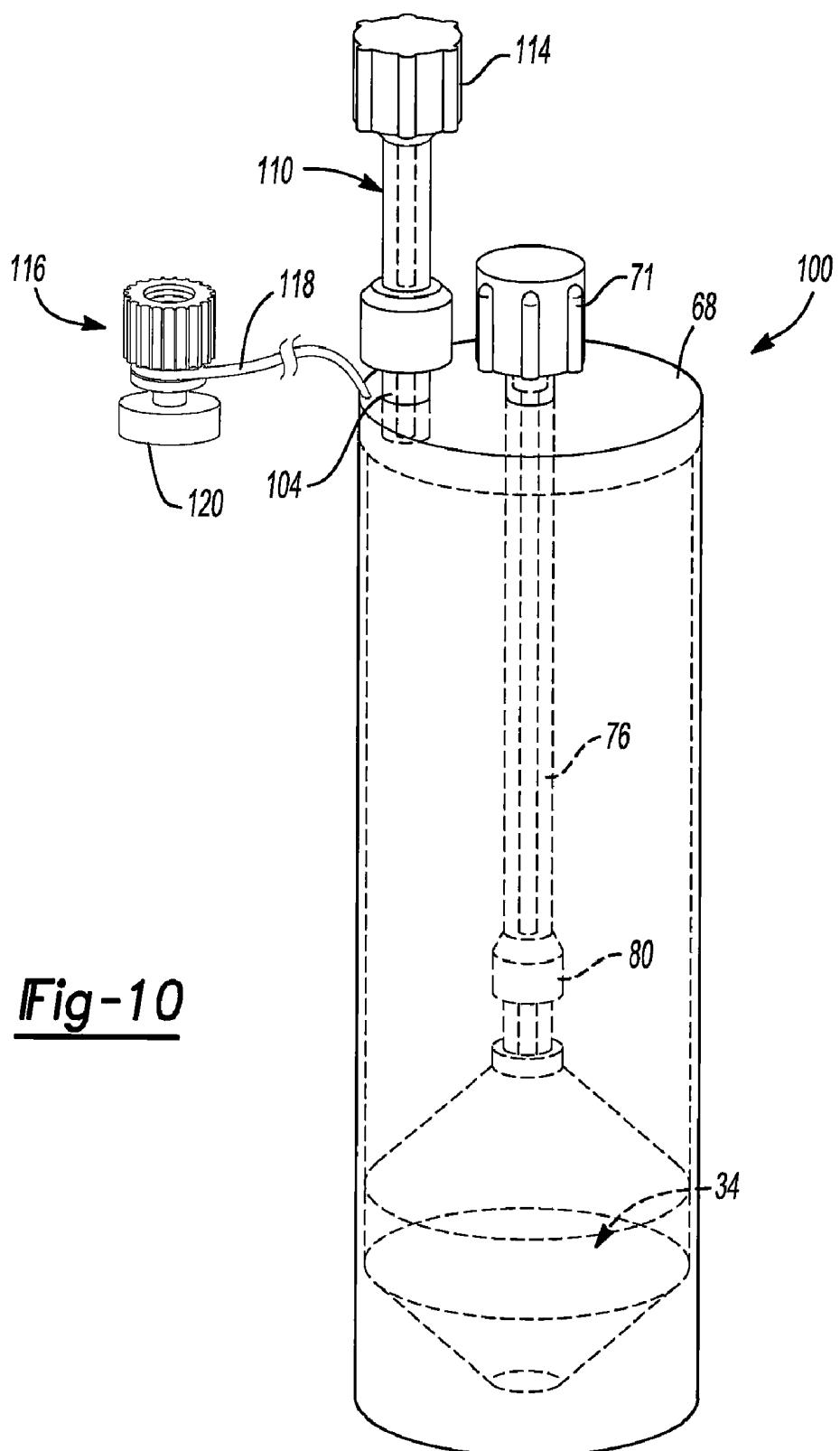


FIG. 8





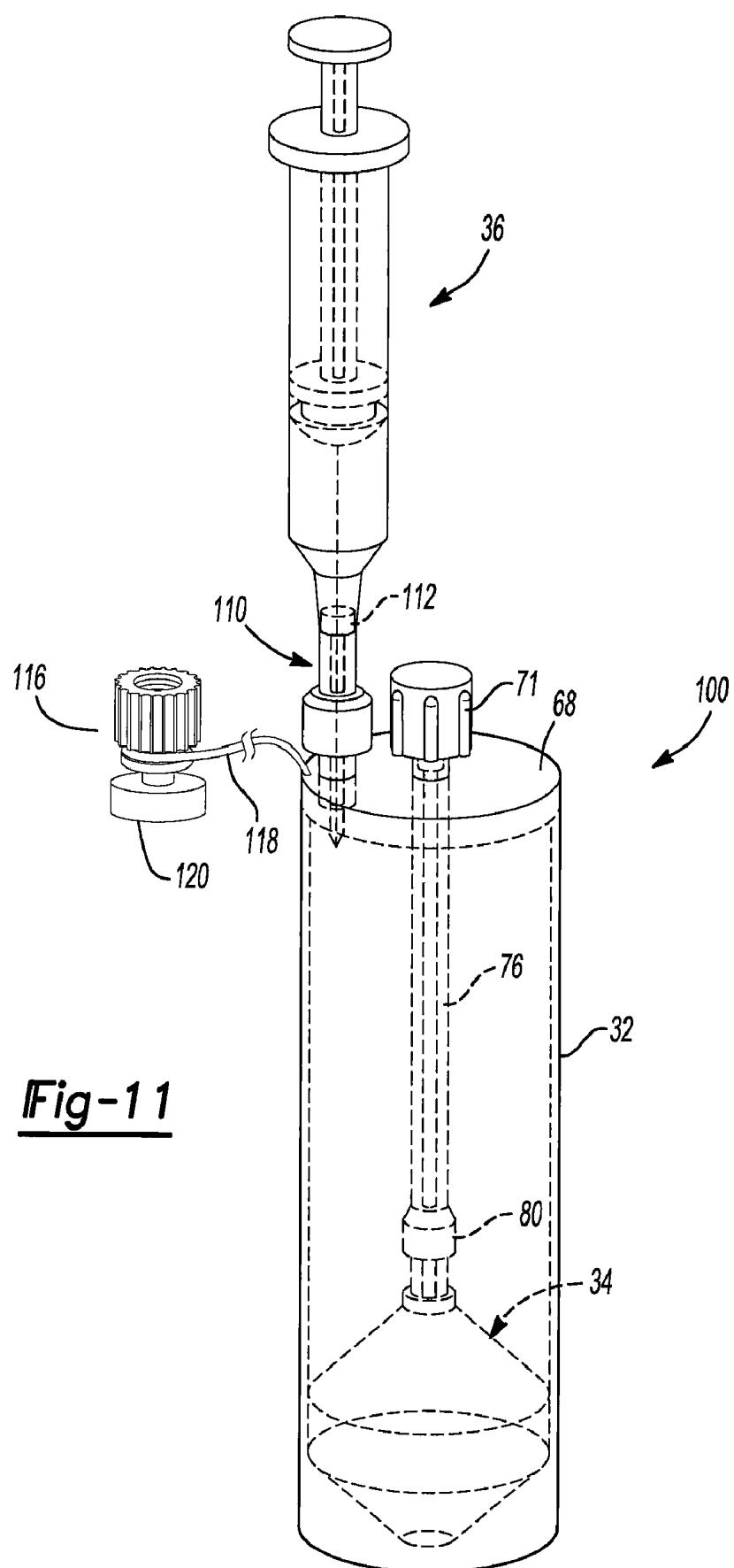


Fig-11

1**METHOD AND APPARATUS FOR COLLECTING BIOLOGICAL MATERIALS****CROSS-REFERENCE TO RELATED APPLICATIONS**

This application is a continuation-in-part Application of U.S. patent application Ser. No. 11/210,005 filed on Aug. 23, 2005 which is now U.S. Pat. No. 7,771,590. This application also claims the benefit of U.S. Provisional Application No. 60/900,758, filed on Feb. 9, 2007. The disclosures of the above applications are incorporated herein by reference.

FIELD

The present teachings relate generally to collection of selected biological materials, in particular to a method and apparatus for separating and collecting a selected biological component.

BACKGROUND

Various biological materials, such as whole blood, adipose tissue and the like, are formed of a plurality of components or fractions. These various fractions can be collected and separated from an anatomy, such as a human anatomy, using various techniques. Nevertheless, generally known techniques may require a plurality of steps and a large volume of biological materials to obtain a selected biological component.

For example, collecting a selected component of whole blood or adipose tissue requires collecting a large sample of whole blood or whole adipose tissue and performing several steps to obtain a selected fraction of the whole sample. It may be desirable to obtain a selected volume for a procedure where time and sample quantity are minimal. Therefore, it may be desirable to provide a method and apparatus to obtain a selected volume of a fraction of a biological material in a short period of time from a selected volume.

SUMMARY

A method and apparatus is provided for obtaining a selected fraction or component of a biological material for a use. The apparatus can generally include a container and a solid or porous piston. A withdrawal tube can be permanently or selectively interconnected with the piston to withdraw a selected fraction of a whole material. Generally, the withdrawal tube can pass through a selected portion of the piston, such as a distal end of the piston to obtain a material that is positioned near a distal portion of the container.

According to various embodiments a system to separate a component from a selected material is disclosed. The system can include a separation container operable to contain the selected material having a top and a bottom and a top wall at a proximal end of the separation container that closes the top of the separation container. A piston can be positioned in the separation container. An injection port can extend through the top wall. In addition, a conduit can be positioned in the separation container operable to remove the selected material from a distal end near the bottom of the separation container past the piston.

According to various embodiments a system to separate a component from a selected material is disclosed. The system can include a container having a side wall, bottom wall, and a top wall and defining an interior volume. An input port can extend from the top wall and define a first passage through the

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top wall to the interior volume. An extraction port can extend from the top wall. A piston can move within the interior volume of the container. In addition, a conduit extending from the extraction port can include a tube extending from the top wall and a passage through the piston.

According to various embodiments, a method of separating a component from a selected material is disclosed. The method can include obtaining the selected material having multiple components and providing a separation system including a tube having a top wall, a piston within the tube, an input port defined through the top wall, an extraction port defined through the top wall, a hollow member extending from the extraction port at least to the piston. The selected material can be positioned in the separation system through the input port with the top wall connected to the tube and between the top wall and the piston. The separation system can be centrifuged while containing the selected material and the piston can move towards the top wall during centrifugation. The component of the selected material can be extracted from past the piston.

Further areas of applicability of the present teachings will become apparent from the detailed description provided hereinafter. It should be understood that the detailed description and various embodiments are intended for purposes of illustration only and are not intended to limit the scope of the teachings.

BRIEF DESCRIPTION OF THE DRAWINGS

30 The present teachings will become more fully understood from the detailed description and the accompanying drawings, wherein:

FIG. 1 is a kit of an apparatus according to various embodiments;

35 FIG. 2 is an environmental view of a separating device according to the various embodiments;

FIG. 3 illustrates the separating device being filled according to various embodiments;

40 FIG. 4 is an environmental view of a filled separating device according to various embodiments;

FIG. 5 is an environmental view of a separating device at a centrifuge according to various embodiments;

FIG. 6 is an environmental view of a separating device after being centrifuged;

45 FIG. 6A is a schematic view of a separating device after being centrifuged;

FIG. 7 is an environmental view of material being withdrawn from the separating device according to various embodiments;

50 FIG. 7A is a schematic view of the piston in the container while material is being withdrawn from the separating device according to various embodiments;

FIG. 8 illustrates the environmental view after a selected component has been withdrawn from the separating device;

55 FIG. 9 is an exploded perspective view of a separation device according to various embodiments;

FIG. 10 is an assembled view of a separation device according to various embodiments; and

60 FIG. 11 is a detail view of a syringe interacting with a separation device according to various embodiments.

DETAILED DESCRIPTION OF VARIOUS EMBODIMENTS

65 The following description of the various embodiments is merely exemplary in nature and is in no way intended to limit the teachings, its application, or uses. Although the following

teachings relate to adipose tissue, it will be understood that the teachings may apply to any appropriate multi-component material, whether biological or not. It will be further understood that a component can be any appropriate portion of a whole, whether differing in density, specific gravity, buoyancy, structure, etc. The component is a portion that can be separated from the whole.

With reference to FIG. 1, a kit 20 can be provided to allow for collection, separation, and application of a selected biological material or component. The kit 20 can be understood to include any appropriate devices or materials, and the following devices are merely exemplary. The kit 20 can include a separation device 30 that can be used to separate a selected material, such as an adipose tissue sample, a whole blood sample, or the like. It will be understood that the separation device 30 can be disposable, reusable, or combinations thereof. For example, the separation device 30 can include a container 32 that may be reusable while a separation piston 34 is not. Further, the kit 20 can include a collection device such as a syringe 36, an application device such as a syringe 38, and a mixing material that may be included in a syringe 40. The mixing material may be any appropriate material such as an anti-clotting agent, a clotting agent, an antibiotic, an enzyme, a buffer, a growth factor or factors, or the like. It will be understood that the kit 20 may also include any other appropriate materials such as bandages, tourniquets, sterilization materials or the like. It will be further understood that the kit 20 may be provided sterilized, prepared for sterilization, or any appropriate combination thereof.

The various syringes 36, 38, 40, may be any generally known syringe. The syringe 36 may also be interconnectable with a needle or cannula 42 that can interconnect with a luer fitting 44 of the syringe 36. The syringe 36 can generally include a container 46 and a plunger 48. This can allow the syringe 36 to withdraw a selected sample, such as an adipose tissue sample from an anatomy, such as a human anatomy, for various purposes. The application syringe 38 can also include a container 50 and a plunger 52. The application syringe 38 can be any appropriate syringe and can be of a size to interconnect with the selected portion of the separation device 30, such as discussed herein. Further, the mixing syringe 40 can also include a container 54 and a plunger 56. The mixing syringe 40 can include any appropriate material, such as those described above. The mixing material provided in the mixing syringe 40. The mixing material can be added to the container 32 at any appropriate time for interaction with the selected material that can also be positioned in the separation container 32.

The separation device 30 includes the container or tube 32 that can include various features. For example, container 32 can be any appropriate size such as 20 ml, 40 ml, 60 ml, any combination thereof, fraction thereof, or any appropriate size. The collection container 32 includes a side wall 60 that can assist in containing the material positioned in the container 32. The tube 32 may also include demarcations 62 that indicate a selected volume.

The sidewall 60 may or may not be flexible under a selected force. For example, the separation device 30 can be positioned in a centrifuge or similar device to apply an increased force of gravity to the material positioned in the tube 32. If the tube 32 is formed of a selected material, the sidewall 60 may flex under the high force of gravity to cause an increased diameter of the tube 32 under the higher force of gravity. Alternatively, the sidewall 60 of the container 32 may be formed of a substantially rigid material that will not flex under a high force of gravity.

The tube 32 further includes a top or proximal portion that defines a cap engaging region 64. The cap engaging region 64 can include a thread or partial threads 66 that can interconnect with a cap 68. The cap 68 can include an internal thread that can thread onto the thread 66 of the top portion 64 to fix the cap 68 relative to the tube 32. Therefore, the cap 68 can be removed from the tube 32, but it will be understood that the cap 68 can also be formed as an integral or single portion of the tube 32. It will be understood that the separating device 30 can be provided as a modular system or can be formed as an integral or unitary member.

Extending through the cap 68 can be a collection or application port 72. The port 72 can include a luer locking portion 74, or any other appropriate interconnection portion. The port 15 72 can also include or be connected to a cap 71. The port 74 can extend through the cap 68 to a withdrawal tube 76. The withdrawal tube 76 may be formed as a single piece with the port 72 or can be interconnectable with the port 72. Further, the withdrawal tube 76 can extend through the piston 34 20 through a central channel 78 defined through the piston 34. The withdrawal tube 76 can define a conduit, such as an extraction conduit. One skilled in the art will understand that a separate tube or cannula can be passed relative to the piston 34 for withdrawal of a material or component of the sample. Thus, the withdrawal tube 76 need not be maintained in the tube 32 for an entire procedure.

The withdrawal tube 76 can, but is not required to, define a piston stop or stop member 80. The stop 80 can act as a stop member for the piston 34 so that the piston 34 is able to move only a selected distance along the withdrawal tube 76. The stop 80 can also be formed by any appropriate portion, such as the sidewall 60. The stop 80 is provided to assist in limiting a movement of the piston 34. Therefore, it will be understood that the withdrawal tube 76 may also act as a rod on which the piston 34 is able to move.

The piston 34 can include any appropriate geometry such as a geometry that substantially mates with the tube 32, particularly a distal end 82 of the tube 32. The distal end of the tube 32 can be flat, conical, tapered, etc. It will be understood, however, that the piston 34 can also include any other appropriate geometry to interact with the tube 32. Further, the piston 34 can include a contacting or central region 84 that includes an outer dimension, such as a circumference or diameter that is generally equivalent to an inner diameter or circumference of the tube 32. Therefore, the piston 34 can contact or engage the sidewall 60 of the tube 32 at a selected time.

The piston 34 can also be formed in any appropriate configuration or of any appropriate material. For example, in addition to the selected geometry of the piston 34, the piston can be porous, non-porous, or include regions of each. For example, the piston 34 can be formed of a porous material such as a screen, a filter, a mesh, or the like. The piston 34, including a porous region, can allow a selected material to pass through and not allow other non-selected materials to pass. The piston 34, therefore, can selectively separate materials or components of a sample.

The middle or tube engaging portion 84 of the piston 34 can include the dimension that is substantially similar to an unchanged or unforced dimension of the wall 60 of the tube 32. For example, it may be formed so that there is substantially little space or a sliding engagement between the tube engaging portion 84 of the piston 34 and the tube 32. However, under a selected force, such as a centrifugal force, the wall 60 of the tube 32 can be compressed axially and be forced outward thereby increasing a dimension, such as a diameter, of the tube 32. The increasing of the diameter of the tube 32

relative to the piston 34 can allow for a freer movement or non-engagement of the tube 32 with the piston 34. In this way, the piston 34 can move relative to the tube 32 or materials can move between the piston 34 and the tube 32.

For example, as discussed herein, the piston 34 may move relative to the tube 32 when the tube is compressed, thus increasing the tube's 32 diameter. The piston 34 can move relative to the withdrawal tube 76, which can allow the piston 34 to move a selected distance relative to the tube 32 or the cap 68. The stop 80, which is provided on the withdrawal tube 76, can assist in selectively stopping the piston 34 relative to the rod 76. This can define a maximum motion of the piston 34 relative to the withdrawal tube 76.

A selected material, such as a biological material, can be positioned in the tube 32 and the tube 32 can be positioned in a centrifuge with the piston 34. During the centrifugal motion, the tube 32 can compress, thereby increasing its diameter relative to the piston 34. The compression can allow the piston 34 to more easily move relative to the withdrawal tube 76 and the container tube 32. Therefore, the piston 34 can assist in separating a selected material positioned in the container tube 32. Once the centrifugal force is removed or reduced, the axial compression of the container tube 32 can be reduced to thereby return it substantially to its original dimensions. As discussed above, its original dimensions can be substantially similar to those of the piston 34, particularly the tube engaging portion 84, which can hold the piston 34 in a selected position relative to the tube 32. This can assist in maintaining a separation of the material positioned in the tube 32, as discussed herein.

It will be understood that the separation system 30 can be used with any appropriate process or various selected biological materials or multi-component materials. Nevertheless, the separation system 30 can be used to separate a selected biological material such as stromal cells, mesenchymal stem cells, blood components, adipose components or other appropriate biological or multi-component materials. Thus, it will be understood that the following method is merely exemplary in nature and not intended to limit the teaching herein.

With additional reference to FIG. 2, a patient 90 can be selected. The patient 90 can include an appropriate anatomy and the collection device 36 can be used to collect a selected portion of biological material. For example, the collection device 36 can engage a portion of the patient 90 to withdraw a selected volume of adipose tissue. The adipose tissue can be selected from any appropriate portion of the anatomy, such as from the abdominal region. In addition, various other components may be withdrawn into the collection tube 36, such as whole blood, stem cells, and the like. Further, the collection device 36 can be a plurality of collection devices that each collect different components, such as one to collect adipose tissue, one to collect whole blood, and others to collect other selected biological materials.

Once the selected biological material is withdrawn into the collection device 36, the biological material 92 can be placed into the tube 32. Once the tube 32 has been filled an appropriate amount with the biological material 92, the piston 34, the rod 76, and the cap 68 can be interconnected with the tube 32.

With additional reference to FIG. 4, the assembled separation device 30 can be pre-treated prior to various other processing steps. For example, selected components, including enzymes, chemicals, antibiotics, growth factors, and the like, can be added to the container tube 32. Further, the selected material, which can include adipose tissue, can be sonicated or treated with a sonic radiation prior to further processing steps. In addition, or alternatively to sonication, various other

agitating methods or devices can be used to mix or agitate the material. For example, a mixing bead, beads, ball, or the like can be placed in the container 32. The container 32 can then be moved with the beads inside to agitate and mix the material. In addition, various rigid arms or extensions can be positioned in the container 32 to assist in agitating or mixing the material.

The sonication of the adipose tissue can perform various steps. For example, the sonication of the adipose tissue can remove or release stromal cells from the adipose tissue cells. It will be understood that sonication of the adipose tissue can be performed at any appropriate time. For example, the sonication of the adipose tissue can be performed once it has been collected into the collection device 36 and prior to being positioned in the tube 32 or after it has been positioned in the tube 32. Further, all of the selected materials, which may include whole blood, various components of whole blood, or the like, can be also added to the tube 32.

With reference to FIG. 5, once the separation system 30 has been optionally pre-processed, such as with agitation and/or sonication, various chemicals, various biologically active materials (e.g. enzymes), it can be positioned in an appropriate separation device, such as a centrifuge 94. The centrifuge 94 can be operated according to any appropriate technique to perform a high gravity separation of the material positioned in the separation device 30. Nevertheless, the centrifuge device can be spun at any appropriate rotation per minute (RPM) such as about 2000 to about 4030 RPMs. This can form a force of gravity on the separation device 30 and the various materials positioned therein of about 740 G's to about 3000 G's. Further, the centrifugation step with the centrifuge device 94 can be performed for any appropriate amount of time. For example, the separation device 30 can be spun at the selected RPMs for about 5 to about 15 minutes. It will be understood that one skilled in the art can determine an appropriate RPM and time setting which can be used to separate selected materials positioned in the separation device 30. Further, the separation of different materials may require different RPMs and different separation times.

As discussed above, the piston 34 can be positioned in the tube 32 to assist in separating the materials positioned in the container tube 32. The piston 34 can be formed of any appropriate materials and according to any appropriate physical characteristics. For example, the piston 34 can be formed of a material or combination of materials that can achieve a selected density. The piston 34 can assist in separating, such as physically separating, selected components of the biological material 92 positioned in the separation device 30. For example, the piston 34 can include a density that is about 1.00 grams per milliliter to about 1.10 grams per milliliter, such as less than about 1.06 grams per cc or 1.06 grams per milliliter. The selected density of the piston 34 can assist in separating denser components or components with a higher specific gravity than the piston 34. For example, stromal cells include a specific gravity that is greater than other components of the biological material 92 positioned in the tube 32 and also greater than that of the piston 34. The piston 34, however, can include any appropriate density.

As discussed above, when the separation device 30 is positioned in the centrifuge 94 the centrifuge 94 can be spun. The forces produced by the centrifuge 94 can compress the container tube 32, which can increase its diameter thus allowing the piston 34 to move relative to the container 32. The various components of the biological material 92 positioned in the separation tube 32 can be physically separated by the piston 34 as it moves relative to the separation tube 32. This can assist in moving at least one of the piston 34 or a portion of the

biological material 92. Though the biological material can originally be positioned on top of the piston 34, the forces and/or flexing of the sidewall 60 can allow at least a component of the material to move past the piston 34. It will be understood, however, that the sidewall 60 may not flex and that the material is simply forced past the piston 34 between the piston 34 and the sidewall 60. Thus, it will be understood that the material can move past the piston 34 to the distal end 82 to container 32 according to any appropriate method such as flexing the sidewall 60, moving between a space between the piston 34 and the sidewall 60, or any other appropriate method.

With additional reference to FIG. 6, the biological material 92 can be separated into a plurality of components that are contained within the separation container 32. For example, a first component 92a can be positioned between the piston 34, such as a distal end of the piston 34a and the distal end of the separation container 82. The first biological component 92a can be any appropriate material, including stromal cells, mesenchymal stem cells or the like. If the biological material 92 positioned within the separation tube 32 includes adipose tissue, then various other components can include a plasma and plasma protein component 92b and a fat and oil component 92c. It will be understood, as illustrated in FIG. 6, that the fat and oil component 92c is generally formed near a proximal end of the tube 32 while the denser stromal cells are formed as a cell button near the distal end 82. Further, it will be understood that various materials, including plasma and plasma proteins, may also include a density that is higher than that of the piston 34 and thus may also be formed or moved towards the distal end 82 of the separation tube 32. Nevertheless, the first component 92a can include a high concentration of the high density materials that is of a selected material to be separated using the separation device 30, because of the piston 34 and the stop 80.

Further, because the various materials, such as plasma or plasma proteins, can include a density that is similar to that of the first component 92a, which can include the stromal cells, the stop 80 can extend from the withdrawal tube 76 to ensure a low concentration or low volume of the plasma, plasma proteins, or the materials that may include a density that is greater than that of the piston 34. Although it may be selected to include a selected volume of the plasma or plasma proteins near the distal end 82 of the separation tube 32, such as for withdrawal of the selected cells, such as stromal cells, it may be selected to keep the concentration at a selected amount. Therefore, the stop 80 or other stop or limiting portion (e.g. a lip or edge in the container 32) can assist in achieving the selected volume and concentration of the first component 92a to be separated by the separation device 30 as the piston 34 moves towards the stop 80, as illustrated in FIGS. 6 and 6A, where the piston 34 is illustrated to have moved away from the distal end 82 of the container 32.

With additional reference to FIG. 7, the withdrawal device 38 can be interconnected with the withdrawal port 72 which interconnects the withdrawal device 38 with the withdrawal tube 76. As discussed above, the withdrawal tube 76 can pass through the piston 34. Because the withdrawal tube 76 can be fixed relative to the cap 78, the withdrawal tube 76 may not move during the centrifugation process. This allows the piston 34 to move relative to the separation tube 32 while the withdrawal tube 76 maintains its position, as illustrated in FIGS. 6, 6A, and 7. The withdrawal tube 76 can include a portion positioned generally near the distal portion 82 of the separation tube 32. Therefore, the withdrawal port 72 can be interconnected or operable to remove a material that is positioned near the distal end 82 of the separation tube 32. Though

the piston 34 can move proximally and allow for separation of a volume near the distal end 82 of the separation tube 32, the withdrawal tube 76 is still positioned near the distal end 82 of the separation tube 32. Therefore, the collection device 38 can be interconnected with the withdrawal port 72 and used to withdraw the volume of material that is positioned near the distal end of the tube 82, as illustrated in FIGS. 6, 6A, and 7. Thus, the separated material, which can include stromal cells or other appropriate biological components, can be withdrawn after being separated and concentrated with the separation system 30. Other various components, such as the components 92b and 92c of the biological material 92 can be retained in the tube 32.

As the collection device 38 withdraws material from the separation tube 32, the piston 34 can be moved generally in the direction of the arrow A, as illustrated in FIGS. 7 and 7A, away from the stop 80. This can allow for a displacement of the volume being removed into the collection tube 38 as the piston 34 moves in the direction of arrow A towards the distal end 82 of the separation tube 32. Further, this movement of the piston 34 can assist in withdrawing the material from the distal end 82 of the separation tube 32.

With reference to FIGS. 7A and 8, the piston 34 can remain or, again, move to substantially fill the internal volume of the distal portion 82 of the separation tube 32 as it moves toward the distal end 82 as the component is withdrawn. Therefore, the piston 34 can also assist in withdrawing the material from the separation tube 32. Since the piston 34 can substantially fill the volume of the material 92a being withdrawn from the separation tube 32, it can help insure that substantially all of the volume of the material 92a is withdrawn from the separation container 32.

Therefore, the separation device 30 can assist in separating, concentrating, and collecting a selected biological component of the biological material 92. It will be understood that while collecting stromal cells from a sonicated adipose tissue is described that the separation, concentration, and collection of any selected biological component may be performed. One skilled in the art will understand that the separation device 30 can be used with any appropriate biological material that can be positioned in the separation tube 32.

The separation device 30 can be used to separate and concentrate a selected volume of material from a substantially small volume of the whole biological material 92. Because the separation system 30 includes the various components, including the withdrawal tube 76 that extends substantially the length of the separation container 32, and the piston 34, the biological material 92 can be effectively separated and concentrated into various components. The denser component 92a can be easily withdrawn from the separation tube 32 without interference of the other components of the biological material 92.

The withdrawn material, which may include the stromal cells, can then be used for various purposes. The withdrawn material can include the selected biological component, such as stromal cells, mesenchymal stem cells, or other stem cells. The stromal cells that are collected from the selected biological material, such as adipose tissue, can be applied to various portions of the anatomy to assist in healing, growth, regeneration, and the like. For example, during an orthopedic procedure, an implant may be positioned relative to a bony structure. The stromal cells or other components can be applied near the site of the implantation, to the implant before implantation, to an area of removed bone, or the like, to assist in regeneration of growth of the bone. The stem cells, such as the stromal or mesenchymal cells, can assist in healing and growth of the resected bone. Therefore, the separated and

concentrated biological component, which can include the stromal cells or other appropriate biological components, can be applied to assist in regeneration, speed healing after a procedure, or other appropriate applications. Briefly, the undifferentiated cells can differentiate after implantation or placement in a selected portion of the anatomy. Alternatively, the cells can release factors that direct the activity of other cells to assist in regeneration, speed healing, or other appropriate applications.

With reference to FIGS. 9 and 10, the kit 20 can include a separation device 100 that is similar to the separation device 30. While the separation device 100 differs from the separation device 30 in various aspects those identical portions will be referenced with identical reference numerals. Briefly, the separation device 100 can include the separation container 32 or tube. Further, the separation device 100 can include the piston 34. The piston 34 can be positioned within the tube 32 of the separation device 100. The separation device 100 can also include the cap or top wall 68. According to various embodiments, the top wall 68 can be substantially fixed to a proximal end 102 of the tube 32. As discussed above, the top wall 68 can also threadably engage a cap engaging region 64 of the tube 32. An adhesive can be used to fix the cap or top wall 68 to the proximal end 102 of the tube 32 or the two can be formed as a single member.

The separation device 100 can differ from the separation device 30 according to various features. For example, the separation device 100 can include an injection port or second port 104. The injection port 104 can extend between an outlet end 106 and an inlet end 108. The inlet end 108 can also include a connection portion, such as a quarter turn or luer connection that can interconnect with an injection port extender 110. The injection port extender 110 can include a top or injection end 112. A cap 114 can be positioned over the top 112 of the extension 110. The top 112 can include a connection portion, such as a luer lock or other connection portion to connect with the cap 114 or an injection syringe, as discussed further herein.

The separation device 100 can also include a second injection port cap 116. The second injection port cap 116 can be tethered to the top wall 68 with a tether 118. The second injection port cap 116 can also include a sterile contact or holding member 120 that can be removed after use. The second injection port cap 116 can include a luer connection or fixation port to connect to the injection port 104 at the top or connection portion 108.

The injection port 104 allows the material to be injected through the top wall 68 into the tube 32. The top wall 68 can, therefore, be fixed to the proximal end 102 of the tube 32 while the material is being injected or delivered to the tube 32. This can allow the multi-component material 92 to be delivered into the tube 32 in an efficient manner and can also maintain the position of the piston 34 near the distal end 82 of the tube 32. Also, any appropriate mixing material can be added at any appropriate time from the syringe 40 or other source. According to various embodiments, the top wall or cap 68 can be removed a small amount and the material 92 can be delivered through the top end or proximal end 102 of the tube 32. Providing the injection port 104, however, can provide a mechanism and port to inject the material into the injection tube without removing the cap 68 from the tube 32.

With additional reference to FIG. 11, the collection device or syringe 36 can be interconnected with the extension 110 that is interconnected with the injection port 104. The collection syringe 36, as discussed above, can be used to collect the multi-component fluid 92. The multi-component fluid 92 can be injected into the tube 32 of the separation device 100. The

separation device 100 can include the top wall 68 substantially fixed to the tube 32. The extraction port 72 can also be positioned relative to the cap 68 and be interconnected with the conduit 76.

The extension 110 can allow the collection syringe 36 to be interconnected with the injection port 104 in a manner that allows access without interference of the extraction port 72. The extension 110, as discussed above, can include the luer connection near the top end 112 of the extension 110 to interconnect with the collection syringe 36. Therefore, the syringe 36 can be efficiently connected to the extension 110 which is connected to the injection port 104.

Once the material is injected into the tube 32 through the injection port 104, the extension 110 can be removed from the injection port 104. After the extension 110 is removed from the injection port 104, the second injection port cap 116 can be interconnected with the injection port 104. The sterile holder 120 on the second injection port cap 116 can be used to effectively maintain sterility between the second injection port cap 116 and the injection port 104. The second injection port cap 116 can be positioned over the injection port 104 during the centrifugation process and the extraction process from the tube 32.

The separation device 100 can be used in a manner substantially identical to the separation device 30, discussed above. It will be understood that the extension 110 is not required, and can be provided according to various embodiments or when selected by a user. Further, the separation device 100 can be included in the kit 20, either with the separation device 30 or as an alternative thereto. Therefore, one skilled in the art will understand, the separation device 100 can be included with the kit 20 and used as the separation device 30 discussed above. In addition the separation devices 30, 100 and the kit 20 can be used in various procedures, such as wound healing, including stromal cells from adipose tissue and other blood components, as taught in U.S. Provisional Application No. 60/900,758, filed on Feb. 9, 2007, incorporated herein by reference.

The teachings are merely exemplary in nature and, thus, variations that do not depart from the gist of the teachings are intended to be within the scope of the teachings. Such variations are not to be regarded as a departure from the spirit and scope of the teachings.

What is claimed is:

1. A system to separate a component from a selected material, comprising:
a separation container operable to contain the selected material having a top and a bottom;
a top wall at a proximal end of said separation container that closes the top of the separation container;
a piston positioned in said separation container between the top and the bottom of the separation container;
an injection port extending through the top wall;
a conduit positioned in said separation container operable to remove the selected material from a distal end near said bottom of said separation container between said piston and said bottom; and
a stop member at a fixed location within said separation container and operable to engage said piston at the fixed location within said separation container,
wherein said stop member is operable to resist a motion of said piston relative to said separation container.
2. The system of claim 1, further comprising:
a centrifuge;
wherein said separation container is operable to be positioned in said centrifuge and said centrifuge is operable to spin said separation container;

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wherein said piston is operable to move within said separation container between said top and said bottom due to a force on said piston during said spin in said centrifuge.

3. The system of claim 1, wherein said conduit includes a tube extending between an extraction port defined in the top wall at the proximal end and said distal end of said separation container.

4. The system of claim 1, wherein said piston includes a density of about 1.00 grams per milliliter to about 1.10 grams per milliliter.

5. The system of claim 1, further comprising: at least one of a collection system, a mixing system, an application system, a withdrawal system, or combinations thereof.

6. The system of claim 1, wherein said stop member is connected to at least one of said conduit or said separation container and is operable to resist a motion of said piston relative to said separation container.

7. The system of claim 3, wherein said piston is operable to move along said conduit; and

wherein said stop member defines a surface to engage said piston to stop a movement of said piston along said conduit.

8. The system of claim 1, further comprising: a port extending through said top wall; wherein said conduit extends through said piston; wherein said conduit and said port operably interconnect to allow withdrawal of the selected component from a position on a side of the piston opposite a side of the piston near of the top wall.

9. A system to separate a component from a selected material, comprising:

a container having a side wall, bottom wall, and a top wall and defining an interior volume;

an input port extending from the top wall and defining a first passage through said top wall to said interior volume;

an extraction port extending from the top wall; a piston operable to move within said interior volume of said container due to a force exerted on said piston during the separation of the component from the selected material;

a conduit extending from said extraction port, including: a tube extending from said top wall; and a passage through said piston;

wherein the conduit allows extraction of the component from a position between said piston and said bottom wall; and

a stop member; wherein the stop member is connected at a fixed location relative to at least one of said tube or said container; wherein said stop member cooperates with said piston to limit a distance said piston is able to move within said interior volume of said container when said piston moves due to the force exerted on said piston during the separation of the component from the selected material.

10. The system of claim 9, further comprising: an input port extender; wherein said input port extender defines a second passage operable with the first passage of the input port to allow access to said interior volume.

11. The system of claim 10, further comprising: an input syringe; wherein said input syringe is operable to be interconnected with at least one of the input port or the input port extender;

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wherein the syringe is operable to cooperate with at least one of the first passage, the second passage, or combinations thereof.

12. The system of claim 9, wherein said input port and said first passage are operable to provide an access to said interior volume between said top wall and said piston.

13. The system of claim 9, further comprising: an extraction syringe, wherein the extraction syringe is operable to be interconnected with said extraction port.

14. The system of claim 13, wherein said extraction syringe is operable with said conduit and said extraction port to extract the component from a position between said piston and said bottom wall after said piston has moved due to the force exerted on said piston during the separation of the component from the selected material.

15. A system to separate a component from a selected material, comprising:

a separation container operable to contain the selected material having a top and a bottom;

a top wall at the top of the separation container that closes the top of the separation container;

an injection portion extending through the top wall; an extraction portion extending though the top wall;

a piston positioned in said separation container operable to move in an area between the top and the bottom of the separation container during a separation of the component from the selected material;

a conduit fixed in said separation container extending from the extraction portion at least towards the piston, wherein the conduit is operable to allow removal of the selected material from near the bottom of the separation container in an area between the piston and the bottom of the separation container; and

a stop member that is connected at a fixed location to at least one of the separation container or the conduit; wherein said conduit includes a tube and a passage in said piston;

wherein said tube is operable to extend into said passage in said piston;

wherein said piston is operable to move along said tube; wherein the stop member is operable to resist a motion of the piston relative to the separation container when the piston moves along said tube.

16. The system of claim 15, further comprising: an injection passage through the top wall whereby the injection portion is operable to allow access to an interior of the separation container; and

an extraction passage through the top wall whereby the extraction portion is operable to allow extraction from near the bottom of the separation container in an area between the piston and the bottom of the separation container.

17. The system of claim 15, further comprising: a centrifuge;

wherein the selected material is operable to be placed in the separation container between the piston and the top of the separation container;

wherein the piston is operable to move towards the top from near the bottom during centrifugation in the centrifuge;

wherein the component is operable to be withdrawn sequentially through the passage in the piston, the conduit, and the extraction portion.



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(45) **Date of Patent:** Mar. 29, 2005

(54) **OSMOTIC DELIVERY SYSTEM HAVING SPACE EFFICIENT PISTON**

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(21) Appl. No.: 10/354,142

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Related U.S. Application Data

(63) Continuation of application No. 09/472,600, filed on Dec. 27, 1999, now Pat. No. 6,544,252.

(60) Provisional application No. 60/114,548, filed on Dec. 31, 1998.

(51) **Int. Cl.⁷** A61K 9/22; A61K 13/00

(52) **U.S. Cl.** 604/892.1; 424/422

(58) **Field of Search** 604/890.1, 891.1, 604/892.1, 130–133, 141, 143, 151, 218; 424/422, 451, 452, 464, 465

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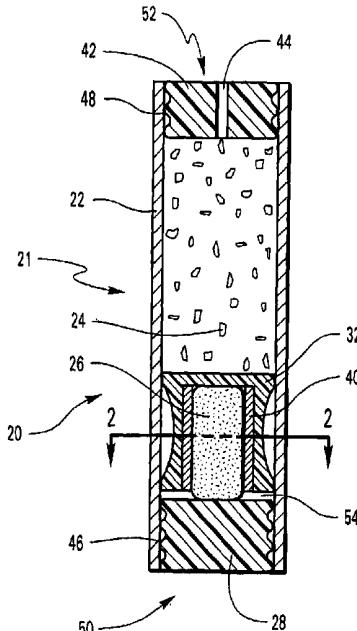
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(57) **ABSTRACT**

An osmotic delivery system having a space-efficient piston is provided. The enclosure has an interior holding the position, a beneficial agent, and an osmotic agent including a tablet. The piston is movable with respect to an interior surface of the capsule, and defines a movable seal with the interior surface of the capsule. The movable seal separates the osmotic agent from the beneficial agent. The piston has a recess that receives at least a portion of the osmotic agent. The osmotic agent imbibes liquid from a surrounding environment through a semipermeable body to cause the piston to move and in turn cause delivery of the beneficial agent from the capsule.

22 Claims, 4 Drawing Sheets



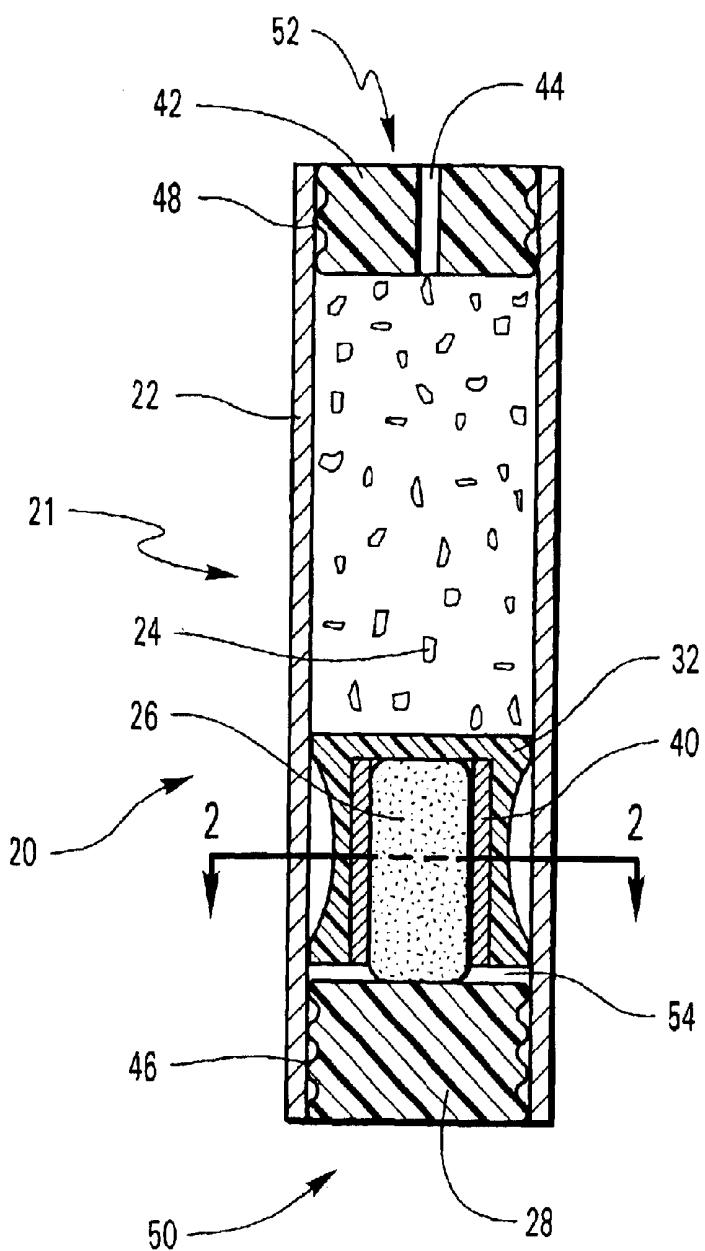


FIG. 1

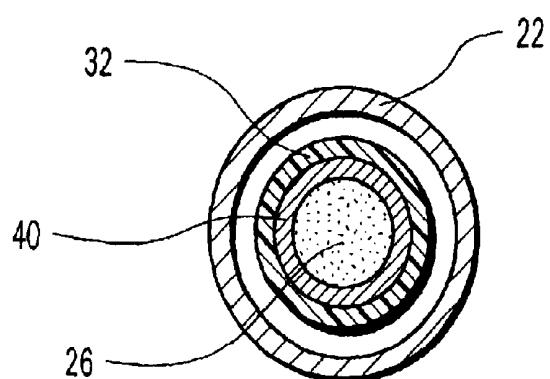


FIG. 2

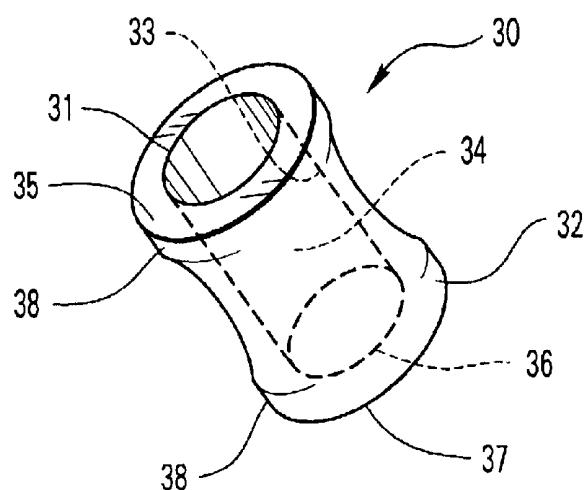


FIG. 3

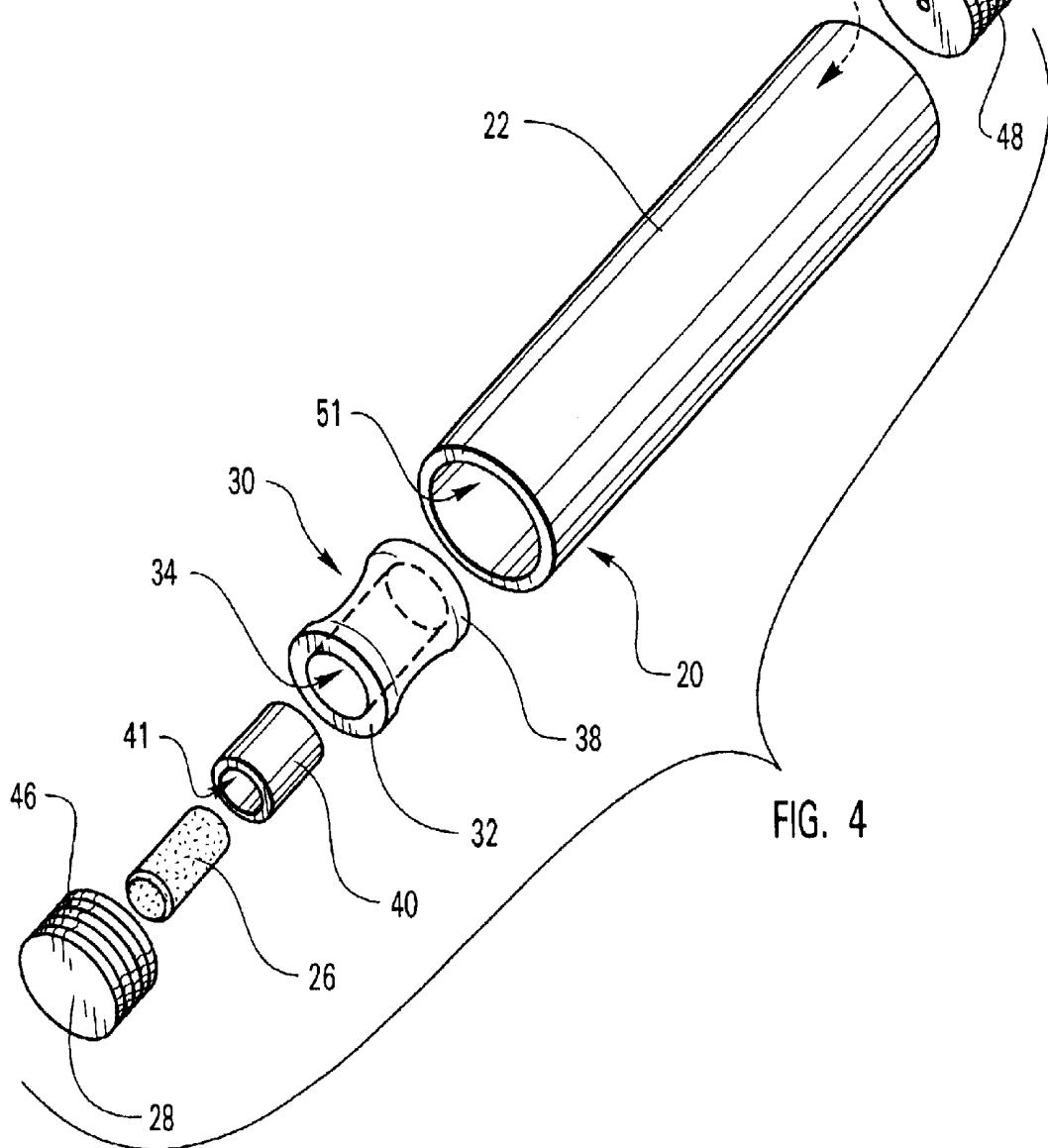


FIG. 4

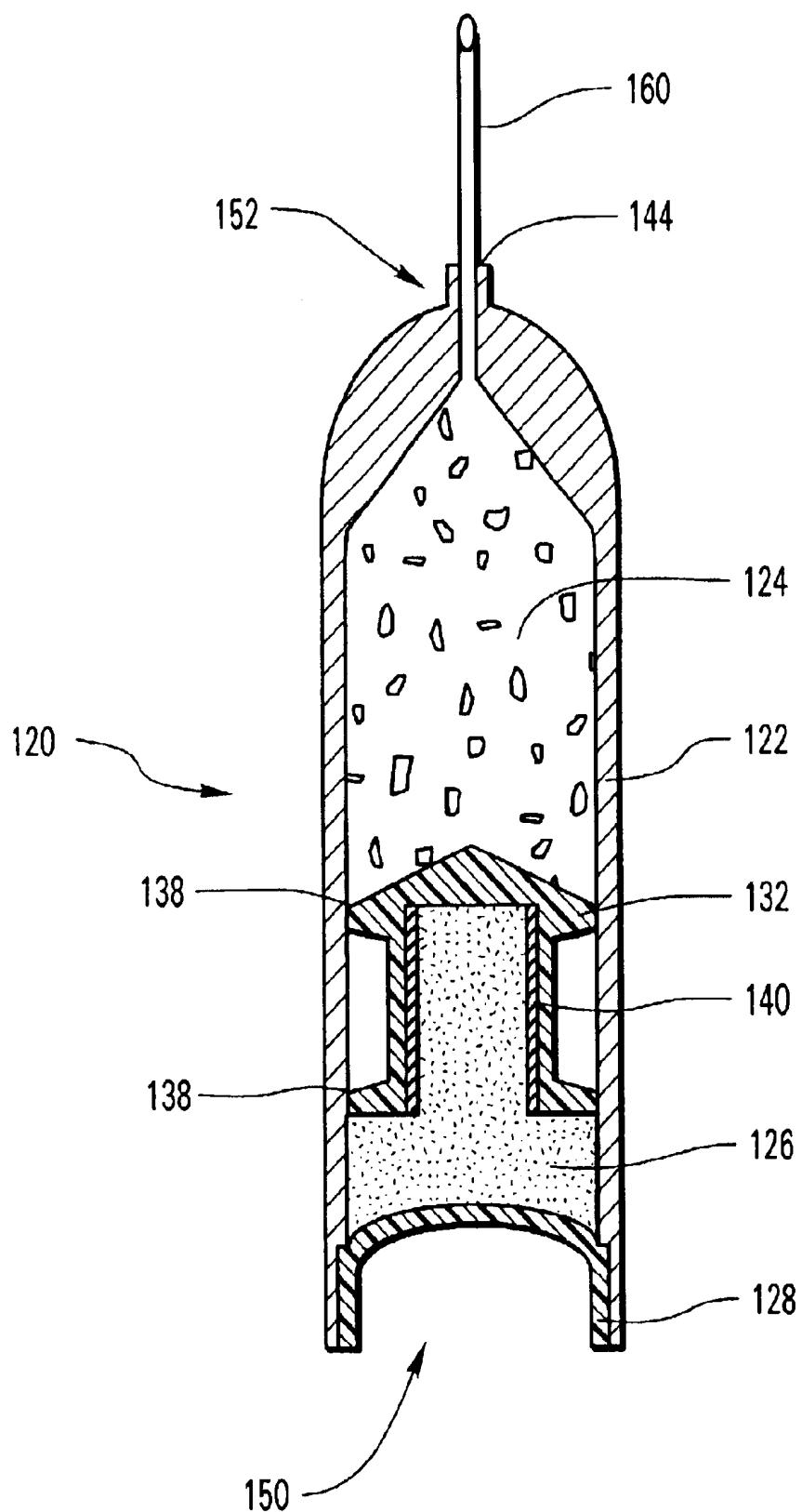
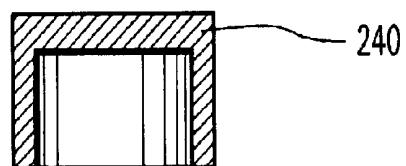
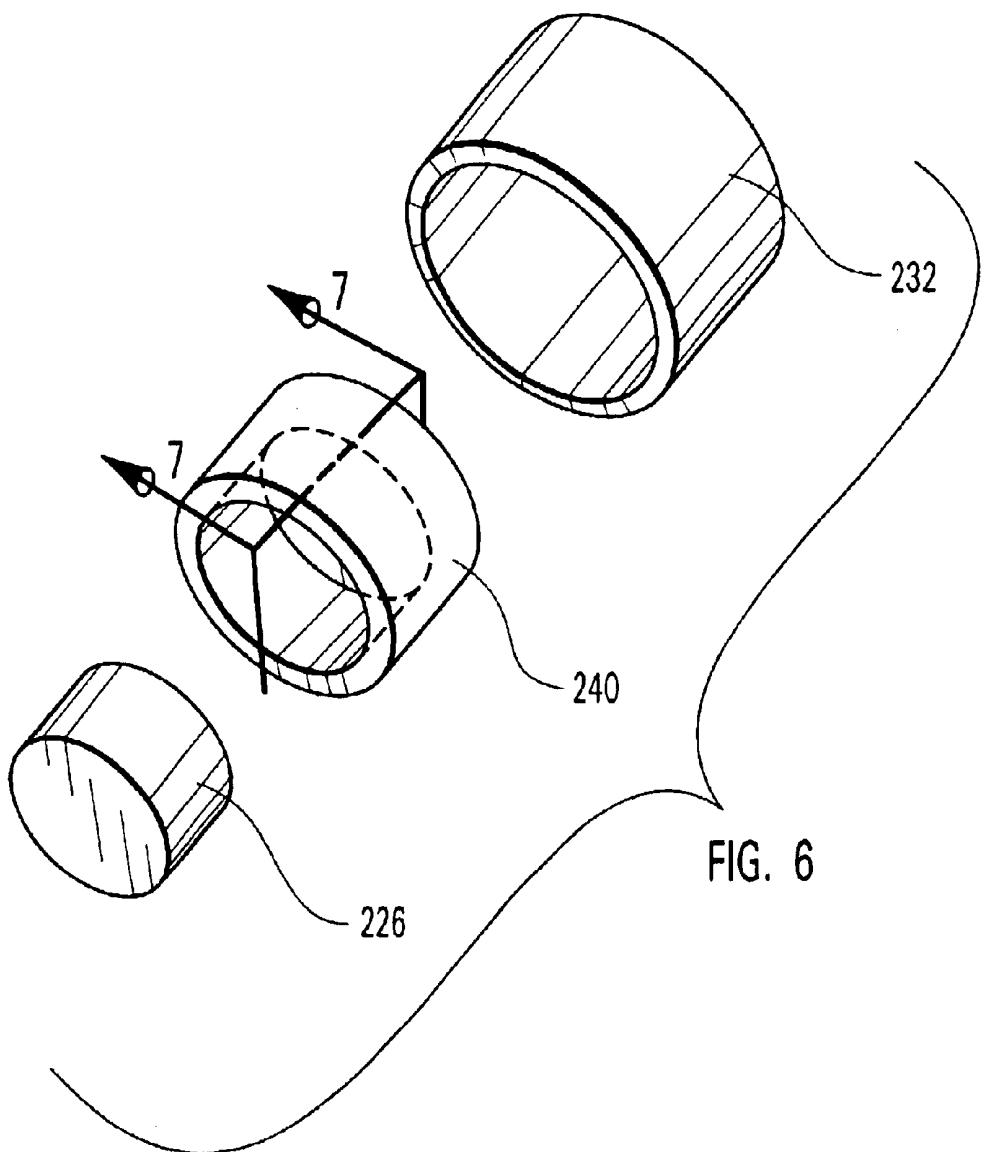


FIG. 5



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**OSMOTIC DELIVERY SYSTEM HAVING
SPACE EFFICIENT PISTON****CROSS-REFERENCE TO RELATED
APPLICATIONS**

The present invention is a continuation of, and claims priority from U.S. patent application Ser. No. 09,472,600, filed Dec. 27, 1999, now U.S. Pat. No. 6,544,252, issued Apr. 8, 2003, which claims the benefit of U.S. Provisional Application Ser. No. 60/114,548, filed on Dec. 31, 1998, the entire disclosures of which are hereby incorporated herein by reference in their entirities.

BACKGROUND OF THE INVENTION**1. Field of the Invention**

The present invention relates to osmotic delivery systems for delivering beneficial agents, and more particularly, to an osmotic delivery system having a piston with a recess for receiving an osmotic agent.

2. Description of the Related Art

Controlled delivery of beneficial agents, such as drugs, in the medical and veterinary fields, has been accomplished by a variety of methods. One method for controlled prolonged delivery of beneficial agents involves the use of osmotic delivery systems. These devices can be implanted to release beneficial agents in a controlled manner over a preselected time or administration period. In general, osmotic delivery systems operate by imbibing liquid from the outside environment and releasing corresponding amounts of the beneficial agent.

A known osmotic delivery system, commonly referred to as an "osmotic pump," generally includes some type of capsule or enclosure having a semipermeable portion that may selectively pass water into an interior of the capsule that contains a water-attracting osmotic agent. In such a known osmotic delivery system, the walls of the capsule are substantially impermeable to items within and outside the capsule, and a plug acts as the semipermeable portion. The difference in osmolarity between the water-attracting agent and the exterior of the capsule causes water to pass through the semipermeable portion of the capsule, which in turn causes the beneficial agent to be delivered from the capsule through the delivery port. The water-attracting agent may be the beneficial agent delivered to the patient. However, in most cases, a separate osmotic agent is used specifically for its ability to draw water into the capsule.

In some instances, a piston is required to separate the beneficial agent from the osmotic agent to prevent the osmotic agent from mixing with or contaminating the beneficial agent. The structure of the capsule is such that the capsule does not expand when the osmotic agent takes in water and expands. As the osmotic agent expands, pressure causes the piston to move and the beneficial agent to be discharged through the delivery orifice at the same rate as the liquid, which is typically water, enters the osmotic agent by osmosis. Osmotic delivery systems may be designed to deliver a beneficial agent at a controlled constant rate, a varying rate, or in a pulsatile manner.

In those osmotic delivery systems that require the use of a piston to separate the beneficial agent and the osmotic agent, the piston necessarily occupies space in the capsule. Hence, if the piston is needed to separate the beneficial agent and the osmotic agent, and the size of the capsule is not changed, the amount of beneficial agent or osmotic agent that can be held within the capsule decreases as compared to

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another osmotic delivery system having the same size capsule that does not include a piston. Decreasing the amount of beneficial agent within the capsule detrimentally decreases the net amount of beneficial agent that can be delivered over a sustained period of time. Decreasing the amount of osmotic agent within the capsule detrimentally decreases the sustained period of time through which continuous delivery of the beneficial agent can be obtained.

But if the specific application requires a specific amount of beneficial agent or osmotic agent that cannot be varied and a piston must be used to separate the beneficial agent from the osmotic agent, the size of the capsule must be increased to accommodate for the extra space occupied by the piston such that the amount of osmotic agent or beneficial agent in the capsule does not vary. While simply increasing the size or volume of the capsule to accommodate for the extra volume occupied by the piston may appear to be a simple solution, because many osmotic delivery systems are destined for implantation in humans or animals, it is especially desirable to decrease the size of the osmotic delivery system as much as possible, while still allowing the osmotic delivery system to deliver the beneficial agent over a prolonged period of time. Additionally, simply increasing the size of the capsule for those applications requiring a piston that separates the beneficial agent from the osmotic agent is inexpedient as it is desirable to use one capsule for multiple osmotic delivery system applications. Moreover, it has been particularly problematic to increase the amount of time over which steady state release of the beneficial agent may be obtained with current osmotic delivery systems incorporating conventional pistons, without increasing the size of the capsule to hold more beneficial agent or osmotic agent. These problems associated with current osmotic delivery systems having known pistons have created a need for a solution.

SUMMARY OF THE INVENTION

Generally speaking, the present invention provides an osmotic delivery system that strives to efficiently utilize the space within the enclosure of the osmotic delivery system.

The present invention strives to address the disadvantages of known osmotic delivery systems by providing an osmotic delivery system having a capsule. The capsule has an interior for holding a beneficial agent. The interior has an interior surface. An osmotic agent is located in the interior of the capsule. A semipermeable body is in liquid communication with the capsule and permits liquid to permeate through the semipermeable body to the osmotic agent. A piston is located within the interior of the liquid impermeable capsule. The piston is movable with respect to the interior surface of the capsule, and defines a movable seal with the interior surface of the capsule. The movable seal defined by the piston separates the osmotic agent from the beneficial agent. The piston has a recess that receives at least a portion of the osmotic agent. The osmotic agent is located between the piston and the semipermeable body. The osmotic agent imbibes liquid from a surrounding environment through the semipermeable body to cause the piston to move and in turn cause delivery of the beneficial agent from the capsule.

In accordance with another aspect of the present invention an osmotic delivery system includes a piston having a recess. An osmotic agent is located within the recess. An enclosure has an interior that holds the piston and the osmotic agent. The piston is movable with respect to the enclosure. The enclosure has a semipermeable body in liquid

communication with the osmotic agent that permits liquid to permeate through the semipermeable body to the osmotic agent. The osmotic agent imbibes liquid from a surrounding environment and causes the piston to move.

According to another aspect of the present invention, an osmotic delivery system includes a capsule having a tubular interior. A semipermeable body is located at least partially within the tubular interior. A piston is located within the tubular interior. The piston has a recess. The piston defines a seal with an interior surface of the tubular interior. The piston is movable with respect to the interior surface of the tubular interior and with respect to the semipermeable body. An osmotic agent is located at least partially in the recess and the tubular interior. A beneficial agent is located within the tubular interior. The piston separates the beneficial agent from the osmotic agent. The semipermeable body is located on the same side of the liquid impermeable piston as the osmotic agent.

Other objects, advantages and features associated with the present invention will become readily apparent to those skilled in the art from the following detailed description. As will be realized, the invention is capable of other and different embodiments, and its several details are capable of modification in various obvious aspects, all without departing from the invention. Accordingly, the drawings and the description are to be regarded as illustrative in nature, and not limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be described in greater detail with reference to the accompanying drawings in which like elements bear like reference numerals, and wherein:

FIG. 1 is a sectional view of an osmotic delivery system according to one embodiment of the present invention;

FIG. 2 is a sectional view of an osmotic delivery system according to the present invention taken along the line 2—2 of FIG. 1;

FIG. 3 is a perspective view of a piston according to one embodiment of the present invention;

FIG. 4 is an exploded perspective view of an osmotic delivery system according to one embodiment of the present invention; and

FIG. 5 is a sectional view of another osmotic delivery system according to the present invention.

FIG. 6 is a perspective view of a cup-shaped sleeve for insertion into a recess of a piston in accordance with another embodiment of the present invention.

FIG. 7 is sectional view of the sleeve of FIG. 6 taken along the line 7—7 of FIG. 6.

DETAILED DESCRIPTION OF THE INVENTION

As shown in FIGS. 1–4, the present invention relates to an osmotic delivery system 20 for delivering a beneficial agent 24. The osmotic delivery system 20 includes a “space-efficient” piston 30. The piston 30 includes a recess 34 that receives an osmotic agent 26. The osmotic delivery system 20 also includes an enclosure 21 that encloses the piston 30 and the osmotic agent 26. The piston 30 is movable within the enclosure 21 and defines a movable seal that substantially prevents the osmotic agent 26 and the beneficial agent 24 from adversely affecting one another. A semipermeable body 28 is in liquid communication with the osmotic agent 26 and permits liquid to permeate through the semipermeable body to the osmotic agent. The osmotic agent 26

imbibes the liquid from a surrounding environment and causes the piston 30 to move, which, in turn, causes the beneficial agent 24 to be released from the osmotic delivery system 20.

The configuration of the osmotic delivery system 20 according to the present invention illustrated in FIGS. 1–4 is one example of an osmotic delivery device and is not to be construed as limiting the present invention. The present invention is generally applicable to all osmotic delivery devices having any number of shapes, and to all such devices administered in any variety of methods, such as oral, ruminal, and implantable osmotic delivery techniques. Such devices may also be placed in reservoirs, tanks, or pools.

The enclosure 21 of the osmotic delivery system 20 encloses or contains the osmotic agent 26 and the body 32 of the piston 30 (shown in FIG. 3). The enclosure 21 includes a tubular or elongated and substantially cylindrical capsule 22 illustrated in FIGS. 1 and 4. The capsule 22 has a first opening 51 at a first end 50 and a second opening 53 at a second end 52 opposite the first end 50. The enclosure 21 also includes the semipermeable body 28 that obstructs, blocks, closes-off, or plugs the first opening 51 in the capsule 22 to enclose the osmotic agent 26 and body 32 of piston 30. Thus, the first opening 51 receives the semipermeable body 28.

The enclosure 21 also includes a delivery port 44 located at the second end 52 of the capsule 22. The delivery port 44 delivers the beneficial agent 24 from the osmotic delivery system 20. According to other embodiments of the present invention, the capsule 22 may take different forms and shapes. For example, the capsule 22 can be tablet-shaped, have an elliptical cross-section, and can be formed from multiple piece tubes or cylinders, or two spheroidal sections. Additionally, the second opening 53 of the capsule 22 can define the delivery port 44, and the first opening 51 can define a channel for communicating a liquid, such as water, from a semipermeable body external of the capsule to an osmotic agent within the capsule. The first opening 51 can also define a channel for communicating a liquid from an external environment to a semipermeable body within the capsule.

The delivery port 44 is an orifice formed by conventional techniques. Included among these methods are mechanical drilling, laser drilling, and molding. The enclosure 21 will contain at least one such delivery port 44, and in most configurations, one delivery port will suffice. However, two or more delivery ports 44 may be present without departing from the present invention. The delivery port 44 may be formed in the capsule 22 itself, such as in the embodiment illustrated in FIG. 5 (shown as 144 and 122 respectively), or may be formed in a separate and distinct plug-like member 42 having means for sealing or ribs 48 extending outwardly from the outer surface thereof for insertion into the second opening 53 of the capsule 22. The delivery port 44 can be other configurations. For example, the delivery port 44 can be a slit orifice, such as that disclosed in U.S. application Ser. No. 09/045,944, the entire disclosure of which is hereby incorporated herein by reference, or a spiral orifice, such as that disclosed in U.S. application Ser. No. 08/595,761, the entire disclosure of which is hereby incorporated herein by reference.

The dimensions of the port 44 in terms of both diameter and length will vary with the type of beneficial agent 24, the rate at which the beneficial agent is to be delivered, and the environment into which it is to be delivered. The considerations involved in determining the optimum dimensions of

the delivery port 44 for any particular enclosure or beneficial agent 24 are the same as those for delivery ports or orifices of enclosures of the prior art, and selection of the appropriate dimensions will be readily apparent to those skilled in the art.

The capsule 22 is formed of a material that is sufficiently rigid to withstand expansion of an osmotic agent 26 without changing size or shape. The capsule 22 is preferably substantially impermeable to fluids in the environment as well as to ingredients contained within the osmotic delivery system 20 such that the migration of such materials into or out of the capsule through the impermeable material of the capsule is so low as to have substantially no adverse impact on the function of the osmotic delivery system 20.

Materials that can be used for the capsule 22 are preferably sufficiently strong to ensure that the capsule will not leak, crack, break, or distort under stresses to which it would be subjected during implantation or under stresses due to the pressures generated during operation of the osmotic delivery system 20.

The capsule 22 can be formed of chemically inert and biocompatible, natural or synthetic materials that are known in the art. The capsule material is preferably a non-bioerodible material that can remain in a patient after use, such as titanium or a titanium alloy, and is largely impermeable to materials within and outside the capsule 22. However, the material of the capsule 22 can alternatively be a bioerodible material that bioerodes in the environment after dispensing the beneficial agent. Generally, preferred materials for the capsule 22 are those acceptable for human implants.

In general, typical materials of construction suitable for the capsule 22 include non-reactive polymers or biocompatible metals or alloys. The polymers include acrylonitrile polymers such as acrylonitrile-butadiene-styrene terpolymer, and the like; halogenated polymers such as polytetrafluoroethylene, polychlorotrifluoroethylene, copolymer tetrafluoroethylene and hexafluoropropylene polyimide; polysulfone; polycarbonate; polyethylene; polypropylene; polyvinylchloride-acrylic copolymer; polycarbonate-acrylonitrile-butadiene-styrene; polystyrene; and the like. Metallic materials useful for the capsule 22 include stainless steel, titanium, platinum, tantalum, gold, and their alloys, as well as gold-plated ferrous alloys, platinum-plated ferrous alloys, cobalt-chromium alloys and titanium nitride coated stainless steel. The capsule 22 can be formed from any of the above-mentioned wall-forming materials by the use of a mold, with the materials applied either over the mold or inside the mold, depending on the mold configuration. Additionally, the capsule 22 can be formed by machining. Any of the wide variety of techniques known in the pharmaceutical industry can be used to form the capsule 22.

The interior of the capsule 22 receives the osmotic agent 26, which in the embodiment of the present invention depicted in FIGS. 1 and 4 is an osmotic tablet. The osmotic agent 26, specifically the osmotic tablet of the embodiment of the present invention illustrated in FIG. 1, drives the osmotic flow of the osmotic delivery system 20. The osmotic agent 26 need not be a tablet; it may be other conceivable shapes, textures, densities, and consistencies and still be within the confines of the present invention. Additionally, more than one osmotic tablet may be used to drive the osmotic flow of the osmotic delivery system 20. When the osmotic delivery system 20 is assembled, the capsule 22 contains the osmotic agent 26.

The osmotic agent 26 is a liquid-attracting agent used to drive the flow of the beneficial agent 24 from the osmotic delivery system 20. The osmotic agent 26 may be an osmagent, an osmopolymer, or a mixture of the two. Species that fall within the category of osmagent, i.e., the non-volatile species which are soluble in water and create the osmotic gradient driving the osmotic inflow of water, vary widely. Examples are well known in the art and include magnesium sulfate, magnesium chloride, potassium sulfate, sodium chloride, sodium sulfate, lithium sulfate, sodium phosphate, potassium phosphate, d-mannitol, sorbitol, inositol, urea, magnesium succinate, tartaric acid, raffinose, and various monosaccharides, oligosaccharides and polysaccharides such as sucrose, glucose, lactose, fructose, and dextran, as well as mixtures of any of these various species.

Species that fall within the category of osmopolymer are hydrophilic polymers that swell upon contact with water, and these vary widely as well. Osmopolymers may be of plant or animal origin, or synthetic, and examples of osmopolymers are well known in the art. Examples include: poly(hydroxy-alkyl methacrylates) with molecular weight of 30,000 to 5,000,000, poly(vinylpyrrolidone) with molecular weight of 10,000 to 360,000, anionic and cationic hydrogels, polyelectrolyte complexes, poly(vinyl alcohol) having low acetate residual, optionally cross-linked with glyoxal, formaldehyde or glutaraldehyde and having a degree of polymerization of 200 to 30,000, a mixture of methyl cellulose, cross-linked agar and carboxymethylcellulose, a mixture of hydroxypropyl methylcellulose and sodium carboxymethylcellulose, polymers of N-vinylactams, polyoxyethylene-polyoxypropylene gels, polyoxybutylene-polyethylene block copolymer gels, carob gum, polyacrylic gels, polyester gels, polyurea gels, polyether gels, polyamide gels, polypeptide gels, polyamino acid gels, polycellulosic gels, carbopol acidic carboxy polymers having molecular weights of 250,000 to 4,000,000, Cyanamer polyacrylamides, cross-linked indene-maleic anhydride polymers, Good-Rite® polyacrylic acids having molecular weights of 80,000 to 200,000, Polyox Polyethylene oxide polymers having molecular weights of 100,000 to 5,000,000, starch graft copolymers, and Aqua-Keeps acrylate polymer polysaccharides.

The osmotic agent 26 may be manufactured by a variety of techniques, many of which are known in the art. In one such technique, an osmotically active agent is prepared as solid or semi-solid formulations and pressed into pellets or tablets whose dimensions correspond to slightly less than the internal dimensions of the respective chambers which they will occupy in the capsule interior. Depending on the nature of the materials used, the agent and other solid ingredients that may be included, can be processed prior to the formation of the pellets by such procedures as ballmilling, calendaring, stirring or rollmilling to achieve a fine particle size and hence fairly uniform mixtures of each.

The beneficial agent 24 may optionally include pharmaceutically acceptable carriers and/or additional ingredients such as antioxidants, stabilizing agents, permeation enhancers, etc. In other embodiments of this invention, the beneficial agent 24 contained in the capsule 22 may include flowable compositions such as liquids, suspension, or slurries, which are typically poured into the capsule after the osmotic agent 26 and the body 32 of the piston 30 have been inserted in the capsule.

Patients to whom beneficial agents 24 may be administered using systems of this invention include humans and animals. The invention is of particular interest for applica-

tion to humans and household, sport, and farm animals, particularly mammals. For the administration of beneficial agents, the devices of the present invention may be implanted subcutaneously or intraperitoneally wherein aqueous body fluids or liquids are available to activate the osmotic agent **26**. Devices of the invention may also be administered to the rumen of humans and ruminant animals, in which embodiment the devices may further comprise a conventional density element for maintaining the device in the rumen for extended periods of time of up to 120 days or longer.

The present invention applies to the administration of beneficial agents in general, which include any physiologically or pharmacologically active substance. The beneficial agent **24** may be any of the agents that are known to be delivered to the body of a human or an animal such as medicaments, vitamins, nutrients, or the like. The beneficial agent **24** may also be an agent that is delivered to other types of aqueous environments such as pools, tanks, reservoirs, and the like. Included among the types of agents that meet this description are biocides, sterilization agents, nutrients, vitamins, food supplements, sex sterilants, fertility inhibitors and fertility promoters.

Drug agents that may be delivered by the present invention include drugs which act on the peripheral nerves, adrenergic receptors, cholinergic receptors, the skeletal muscles, the cardiovascular system, smooth muscles, the blood circulatory system, synaptic sites, neuroeffector junctional sites, endocrine and hormone systems, the immunological system, the reproductive system, the skeletal system, autacoid systems, the alimentary and excretory systems, the histamine system and the central nervous system. Suitable agents may be selected from, for example, proteins, enzymes, hormones, polynucleotides, nucleoproteins, polysaccharides, glycoproteins, lipoproteins, polypeptides, steroids, analgesics, local anesthetics, antibiotic agents, anti-inflammatory corticosteroids, ocular drugs and synthetic analogs of these species.

Examples of drugs that may be delivered by devices according to this invention include, but are not limited to, prochlorperazine edisylate, ferrous sulfate, aminocaproic acid, mecamylamine hydrochloride, procainamide hydrochloride, amphetamine sulfate, methamphetamine hydrochloride, benzamphetamine hydrochloride, isoproterenol sulfate, phenmetrazine hydrochloride, bethanechol chloride, methacholine chloride, pilocarpine hydrochloride, atropine sulfate, scopolamine bromide, isopropamide iodide, tridihexethyl chloride, phenformin hydrochloride, methylphenidate hydrochloride, theophylline cholinate, cephalexin hydrochloride, diphenidol, meclizine hydrochloride, prochlorperazine maleate, phenoxybenzamine, thiethylperazine maleate, anisindone, diphenedione erythrityl tetranitrate, digoxin, isofl uorophate, acetazolamide, methazolamide, ebendroflumethiazide, chloropromazine, tolazamide, chlormadinone acetate, phenaglycodol, allopurinol, aluminum aspirin, methotrexate, acetyl sulfisoxazole, erythromycin, hydrocortisone, hydrocorticosterone acetate, cortisone acetate, dexamethasone and its derivatives such as betamethasone, triamcinolone, methyltestosterone, 17-S-estradiol, ethinyl estradiol, ethinyl estradiol 3-methyl ether, prednisolone, 17- α -hydroxyprogesterone acetate, 19-norprogesterone, norgestrel, norethindrone, norethisterone, norethiederone, progesterone, norgestosterone, norethynodrel, aspirin, indomethacin, naproxen, fenoprofen, sulindac, indoprofen, nitroglycerin, isosorbide dinitrate, propranolol, timolol, atenolol, alprenolol, cimetidine, clonidine,

imipramine, levodopa, chlorpromazine, methyldopa, dihydroxyphenylalanine, theophylline, calcium gluconate, ketoprofen, ibuprofen, cephalexin, erythromycin, haloperidol, zomepirac, ferrous lactate, vincamine, diazepam, phenoxybenzamine, diltiazem, milrinone, capropril, mandol, quanbenz, hydrochlorothiazide, ranitidine, flurbiprofen, fufenen, fluprofen, tolmetin, alclofenac, mefenamic, flufenamic, difuinal, nimodipine, nitrendipine, nisoldipine, nicardipine, felodipine, lidoflazine, tiapamil, gallopamil, amlodipine, mioflazine, lisinopril, enalapril, enalaprilat, captorpril, ramipril, famotidine, nizatidine, sucrlafate, etintidine, tetratolol, minoxidil, chlordiazepoxide, diazepam, amitriptyline, and imipramine. Further examples are proteins and peptides which include, but are not limited to, insulin, colchicine, glucagon, thyroid stimulating hormone, parathyroid and pituitary hormones, calcitonin, renin, prolactin, corticotrophin, thyrotropic hormone, follicle stimulating hormone, chorionic gonadotropin, gonadotropin releasing hormone, bovine somatotropin, porcine somatotropin, oxytocin, vasopressin, GRF, prolactin, somatostatin, lypressin, pancreozymin, luteinizing hormone, LHRH, LHRH agonists and antagonists, leuprolide, interferons, interleukins, growth hormones such as human growth hormone, bovine growth hormone and porcine growth hormone, fertility inhibitors such as the prostaglandins, fertility promoters, growth factors, coagulation factors, human pancreas hormone releasing factor, analogs and derivatives of these compounds, and pharmaceutically acceptable salts of these compounds, or their analogs or derivatives.

The beneficial agent **24** can be present in this invention in a wide variety of chemical and physical forms, such as solids, liquids and slurries. On the molecular level, the various forms may include uncharged molecules, molecular complexes, and pharmaceutically acceptable acid addition and base addition salts such as hydrochlorides, hydrobromides, acetate, sulfate, laurylate, oleate, and salicylate. For acidic compounds, salts of metals, amines or organic cations may be used. Derivatives such as esters, ethers and amides can also be used. A beneficial agent can be used alone or mixed with other agents.

Osmotic delivery systems according to the present invention are also useful in environments outside of physiological or aqueous environments. For example, the osmotic delivery system may be used in intravenous systems (attached to an IV pump or bag or to an IV bottle, for example) for delivering beneficial agents to an animal or human. Osmotic delivery systems according to the present invention may also be utilized in blood oxygenators, kidney dialysis and electrophoresis, for example. Additionally, devices or systems of the present invention may be used in the biotechnology area, such as to deliver nutrients or growth regulating compounds to cell cultures. In such instances, activating mechanisms such as mechanical mechanisms are particularly useful.

The osmotic delivery system **20** also includes the aforementioned semipermeable body **28**, such as the semipermeable plug illustrated in FIGS. 1 and 4. The semipermeable body **28** is formed of a semipermeable material that allows liquid to pass from an exterior environment of use into the capsule **22** to cause the osmotic agent **26** to swell. But the material forming the semipermeable body **28** is largely impermeable to the materials within the enclosure and other ingredients within the environment of use. As illustrated in FIG. 1, the semipermeable body **28** is in the shape of a plug that is inserted into the first opening **51** of the capsule **22** at

the first end 50. The semipermeable body 28 defines part of the enclosure 21 because it closes-off the first opening 51 of the capsule 22. Alternatively, the semipermeable body 28 may be located distant from the enclosure 21, but communicate liquid from a surrounding environment of use to the osmotic agent 26 through a tube in liquid communication with the capsule 22 or through other means for communicating liquid. The semipermeable body 28 may also be a membrane coating on the exterior surface of the capsule 22 or a sleeve or cap that slides over a portion of the capsule 22 to enclose the osmotic agent 26.

As shown in FIG. 1, the osmotic delivery system 20 includes the semipermeable body 28, such as the semipermeable plug illustrated. The semipermeable body 28 is typically cylindrically shaped, and has means for sealing or ribs 46 extending outwardly from the outer surface of the semipermeable body. The ribs 46 are the means by which the semipermeable plug operates like a cork or stopper, obstructing and plugging the opening 51 in the capsule 22 of the osmotic delivery system 20 illustrated in FIG. 1. The means for sealing 46 may be the exemplary ribs, or may be other configurations such as threads, a tight interference fit between an outer sealing surface of the plug and the capsule 22, glue, adhesives, ridges, lips, or other devices which join the semipermeable body 28 with the capsule 22 to prevent leakage. The semipermeable body 28 is, therefore, intended for at least partial insertion into an opening of the capsule 22, and the means for sealing 46 the environment of use from an inside of the capsule 22 prevents liquid and other substances in the environment of use, besides the permeation liquid, from entering the osmotic delivery system 20 while also preventing materials from the inside of the delivery system from leaking or escaping to the environment of use.

The semipermeable body 28 is made from a semipermeable material. The semipermeable material of the body 28 allows liquids, especially water, to pass from an exterior environment of use into the capsule 22 to cause the osmotic agent 26 to swell. However, the semipermeable material forming the semipermeable body 28 is largely impermeable to the materials within the capsule 22 and other ingredients within the fluid environment.

Semipermeable compositions suitable for the semipermeable body 28 are well known in the art, examples of which are disclosed in U.S. Pat. No. 4,874,388, the entire disclosure of which is incorporated herein by reference. Such possible semipermeable materials from which the body 28 can be made include, but are not limited to, for example, Hytrel polyester elastomers (DuPont), cellulose esters, cellulose ethers and cellulose ester-ethers, water flux enhanced ethylene-vinyl acetate copolymers, semipermeable membranes made by blending a rigid polymer with water-soluble low molecular weight compounds, and other semipermeable materials well known in the art. The above cellulosic polymers have a degree of substitution, D.S., on the anhydroglucose unit, from greater than 0 up to 3 inclusive. By, "degree of substitution," or "D.S.", is meant the average number of hydroxyl groups originally present on the anhydroglucose unit comprising the cellulose polymer that is replaced by a substituting group. Representative materials include, but are not limited to, one selected from the group consisting of cellulose acylate, cellulose diacetate, cellulose triacetate, mono-, di-, and tricellulose alkanylates, mono-, di-, and tricellulose aroylates, and the like. Exemplary cellulosic polymers include cellulose acetate having a D.S. up to 1 and an acetyl content up to 21%; cellulose acetate having a D.S. of 1 to 2 and an acetyl content of 21% to 35%; cellulose acetate having a D.S. of 2 to 3 and an acetyl content

of 35% to 44.8%, and the like. More specific cellulosic polymers include cellulose propionate having a D.S. of 1.8 and a propionyl content of 39.2% to 45% and a hydroxyl content of 2.8% to 5.4%; cellulose acetate butyrate having a D.S. of 1.8 and an acetyl content of 13% to 15% and a butyryl content of 34% to 39%; cellulose acetate butyrate having an acetyl content of 2% to 29%, a butyryl content of 17% to 53% and a hydroxyl content of 0.5% to 4.7%; cellulose acetate butyrate having a D.S. of 1.8, and acetyl content of 4 average weight percent and a butyryl content of 51%; cellulose triacylates having a D.S. of 2.9 to 3 such as cellulose trivalerate, cellulose trilaurate, cellulose tripalmitate, cellulose trisuccinate, and cellulose trioctanoate; cellulose diacylates having a D.S. of 2.2 to 2.6 such as cellulose disuccinate, cellulose dipalmitate, cellulose dioctanoate, cellulose dipentate; coesters of cellulose such as cellulose acetate butyrate and cellulose, cellulose acetate propionate, and the like.

Other materials for the semipermeable body 28 are polyurethane, polyetherblockamide (PEBAX, commercially available from ELF ATOCHEM, Inc.), injection-moldable thermoplastic polymers with some hydrophilicity such as ethylene vinyl alcohol (EVA). The composition of the semipermeable body 28 is permeable to the passage of external liquids such as water and biological liquids, and it is substantially impermeable to the passage of beneficial agents, osmopolymers, osmagents, and the like.

The osmotic delivery system 20 also includes the movable space-efficient piston 30. The piston 30 is a member that is matingly received by the hollow interior of the capsule 22 and moves when subjected to pressure from the osmotic agent 26 to displace or move the beneficial agent 24. The piston 30 forms a movable seal with the interior surface of the capsule 22. The movable seal formed by the piston 30 separates the osmotic agent 26 and the beneficial agent 24 such that the osmotic agent 26 does not substantially leak or seep past the piston seal and adversely affect the function of the beneficial agent. Hence, the osmotic agent 26 is separated from the beneficial agent 24 by the movable piston 32.

As illustrated in FIG. 3, the body 32 of the piston 30 is a substantially cylindrical member that is configured to fit in the capsule 22 in a sealing manner that allows the piston to slide within the capsule in the longitudinal direction of the capsule. That is, the exterior surface of the piston body 32 abuts against and slides relative to the interior cylindrical surface of the capsule 22. Because the semipermeable body 28 is lodged within the first opening 51, the piston also moves relative to the semipermeable body 28.

The piston body 32 includes annular ring-shaped protrusions or ribs 38 that define the movable or sliding seal with the inner surface of the capsule 22. The ribs 38 are the most outwardly radial surface of the piston body 32. The ribs 38 are the means by which the piston 30 forms a seal with the interior surface of the capsule 22. Thus, the outermost radial diameter of the piston body 32 is greater than the inner diameter of the capsule 22. Although the piston body 32 illustrated in FIG. 3 includes two ribs, other pistons according to the present invention may include one or more ribs. Additionally, the piston body 32 need not include ribs. For example, the exterior surface of the piston body can be entirely cylindrical such that the entire cylindrical exterior surface of the piston body effects a seal with the interior surface of the capsule 22. However, the ribs 38 are preferred as they effect a better movable seal with the interior surface of the capsule 22, as compared to a piston body having an exterior surface that is entirely cylindrical. The piston body 32 is preferably formed of an impermeable resilient and inert

material. In general, materials suitable for the piston body 32 are elastomeric materials including the non-reactive polymers listed above in reference to the materials for the capsule 22, as well as elastomers in general, such as polyurethanes and polyamides, chlorinated rubbers, styrene-butadiene rubbers, and chloroprene rubbers.

As illustrated in FIG. 3, the piston body 32 includes a hollow interior portion or recess 34, such as the cylindrical cavity illustrated. The recess 34 can be other configurations such as a square cavity, concave indentation, conical pit, cup, gouge, depression, or similar space adapted to receive the osmotic agent 26. The recess 34 has a cylindrical and longitudinal interior surface 33 that begins at an insert opening 31 formed by the recess 34 in the first end 35 of the piston body 32, and ends at a depth surface 36 within the body 32 close to the second end 37 of the body 32. Because of the general cylindrical shape of the outer surface of the piston body 32 and the cylindrical shape of the recess 34, the piston is thimble or cup-shaped such that a "bottom of the cup" has a thickness. Because the piston 30 separates the beneficial agent 24 and the osmotic agent 26, the recess 34 preferably does not pierce completely through the piston body 32. The piston body 32 is cup-shaped because the recess 34 defines a hollow area within the piston body 32.

The longitudinal axis of the recess 34 is approximately parallel to the longitudinal axis of the capsule 22, and is preferably coincident with the longitudinal axis of the capsule 22. Additionally, the opening 31 of the recess 34 faces away from the delivery port 44, i.e., toward the semipermeable body 28. The depth surface 36 of the recess preferably extends past the median of the piston body 32 along the longitudinal axis of the piston as measured from the first end 35. The diameter of the recess 34 is typically 50%, preferably greater than 60%, and preferably less than 80% of the inner diameter of the capsule 22. By increasing the diameter of the recess 34, the wall thickness of the piston body 32 decreases. It is preferable that the recess 34 occupy as much internal volume of the piston 30 as possible without destroying the effectiveness of the piston seal when the piston 30 is inserted into the capsule 22. Additionally, the exterior surface of the piston body 32 can take other shapes, such as a chevron or cantilever shape.

Although the cylindrical configuration of the recess 34 is preferred, other configuration recesses fall within the confines of the present invention. For example, the recess 34 or hollow interior portion may be square, rectangular, octagonal, triangular, oval, half-circular, circular, or a shape that matches the shape of the exterior surface of the piston body 32. Likewise, the hollow interior portion 34 may be a series or plurality of recesses, tubes, slots, or gaps within the interior of the piston body 32. All of the above, and other configurations, would function to receive a portion of the osmotic agent 26 such that the piston 30 occupies less space within the capsule 22.

The recess 34 of the piston body 32 receives the osmotic agent 26, such as the osmotic tablet illustrated in FIG. 1. Additionally, the recess 34 also matingly receives an insert or sleeve 40, such as the cylindrical tube illustrated in FIGS. 1 and 2. The sleeve 40 is preferably made from a rigid and impermeable material such as that used for the capsule 22, and helps effect a movable seal between the piston and the interior surface of the capsule 22. For example, the sleeve 40 can be formed from polycarbonate, polysulfone, polystyrene, or an acetal such as DELRIN®(DuPont). The sleeve 40 also can be made out of an inert metal such as stainless steel or titanium. The sleeve 40 is inserted into the recess 34 and has an outer diameter that at least matches the

diameter of the recess 34. Because the recess 34 receives the sleeve 40, it is preferable that the shape of the exterior surface of the sleeve 40 matches or corresponds to the shape of the recess 34. For example, the recess 34 and the sleeve 40 are both cylindrical. It is also preferable that the wall-thickness of the sleeve 40 be thin so as to occupy little space within the recess 34. In general, the wall-thickness of the sleeve 40 must be thick enough to impart enough rigidity to the sleeve to maintain the piston seal with the interior surface of the capsule 22.

The sleeve 40 is sized such that the recess 34 matingly receives the sleeve 40. In instances where it is desirable to increase the outer diameter of the piston body 32, if the piston body 32 is formed of a resilient material, the outer diameter of the sleeve 40 may be greater than the diameter of the recess 34 such that the piston body 32 deflects radially and outwardly when the sleeve 40 is inserted therein. In the embodiment illustrated in FIG. 1, the longitudinal length of the sleeve 40 is substantially equal to the longitudinal depth of the recess 34 in the piston body 32.

It will be appreciated that the sleeve 40 may be in any number of different shapes and sizes, but preferably matches the shape and size of the recess 34 into which the sleeve 40 is inserted. For example, the sleeve 40 may be cup-shaped or shaped like a chevron. In general, the sleeve 40 stabilizes the dimensions and sealing forces of the piston body 32 as the piston moves, especially if an osmotic tablet is used that dissolves into a fluid during operation of the osmotic delivery system 20. Additionally, the sleeve 40 helps prevent the beneficial agent 24 from diffusing into the osmotic agent 26 during storage of the osmotic delivery device 20.

The sleeve 40 is preferably inserted into the recess 34 for assisting the piston body 32 in effecting a movable seal with the interior surface of the capsule 22. Because the piston body 32 is preferably flexible and resilient, the wall of the piston body 32 flexes toward the interior of the recess 32 after the piston body 32 is inserted into the capsule 22. By inserting the preferably rigid sleeve 40 into the opening 31 of the recess 34 such that the sleeve 40 is matingly received, the wall of the piston body 32 will not overly flex inwardly toward the recess 34, and the seal formed between the outer surface of the piston 30 and the interior surface of the capsule 22 is maintained.

FIGS. 6 and 7 illustrate another embodiment of a sleeve. As shown by FIGS. 6 and 7, the sleeve 240 is in the shape of cup, such as a cap or thimble. The sleeve 240 is inserted into the piston 232, and the osmotic agent 226 is inserted in the recess formed by the cup-shaped sleeve 240. The sleeve 240 may be fabricated from an inert and rigid material to ensure that the piston is impermeable.

Although the piston 30 illustrated in FIG. 1 includes the sleeve 40, in some instances, it may not be necessary to include the sleeve 40 in the recess 34 as the material of the piston body 32 is sufficiently rigid to effect a satisfactory seal between the interior surface of the capsule 22 and the piston body 32. In this case, the sleeve 40 need not be inserted into the recess 34. Generally, the wall thickness and the structural characteristics of the piston body 32 determine whether or not a rigid sleeve 40 is needed to assist in defining the seal, which is determinable by experimental methods.

The osmotic agent 26 is at least partially located within the recess 34. Preferably, the majority of the total weight of the osmotic agent 26 is located within the recess 34. The osmotic agent 26 may be completely located within the recess 34, or may extend partially from the recess 34. As

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illustrated in FIG. 1, the volume of the osmotic agent 26 is greater than that of the recess 34 such that the osmotic agent extends from the recess 34 and into a gap or space 54 located between the piston 30 and the semipermeable body 28. The osmotic agent 26 may completely fill the gap 54 such as shown in FIG. 5, or only partially fill the gap 54, as shown in FIG. 1.

The piston body 32 is preferably injection molded. However, the piston body 32 may be fashioned by a different process. For example, the piston body 32 may also be made from extrusion, reaction injection molding, rotational molding, thermoforming, compression molding, and other known processes. If an injection molding process is used to form the piston body 32, the ejector pin or core may be used to form the recess 34, and different length and sized ejector pins or cores may be easily changed to fashion different size recesses 34 to controllably vary the amount of osmotic agent that is received by the recess 34 of the piston 30. Additionally, the recess 34 may be formed in the piston body 32 after the piston body has been formed without a recess. For example, a cylinder of material may be fabricated and sliced into smaller cylinders. Thereafter, a cylindrical section may be removed from the piston body to form the recess 34 in the piston body 32.

Furthermore, the piston body 32 need not be the unitary structure illustrated in FIG. 3. A cylindrical tube may be attached to a flat circular disk to define the cup-shape of the piston 30. Additionally, the sleeve 40 may be cup-shaped, and a resilient tube with ribs may wrap around an outer cylindrical surface of the sleeve to define the piston 30.

It is preferable that the piston body 32 be substantially impervious to liquids, such that the osmotic agent and the liquid that permeates through the semipermeable body 28 does not diffuse through the piston body 32 and affect the beneficial agent 24 located on the side of the piston 30 opposite from that of the osmotic agent 26, and such that the beneficial agent does not diffuse through the piston body 32 and affect the performance of the osmotic agent 26.

Because the recess 34 of the piston body 32 at least receives a portion of the osmotic agent 26, the total volume of the osmotic delivery system 20, as compared to past systems, may be efficiently utilized. That is, rather than locating the osmotic agent 26 entirely between a semipermeable body and a known piston having no recess, the osmotic agent is at least partially located within the piston such that the space within the capsule 22 is efficiently utilized. The space-efficient piston 30 occupies less space in the capsule 22 of the osmotic delivery system 20, as compared to conventional pistons. Because the piston 30 occupies less space within the capsule 22, the internal volume of the interior of the capsule 22 need not be overly increased, if increased at all, to accommodate for the extra space occupied by the piston such that the amount of osmotic agent or beneficial agent in the capsule does not excessively vary when the piston 30 is used. This characteristic of the osmotic delivery system 20 increases the amount of time over which steady-state release of the beneficial agent 24 may be obtained as compared to past osmotic delivery systems that include conventional pistons. Additionally, because the piston 30 occupies less volume than past pistons, the total internal volume of the capsule 22 of the osmotic delivery system 20 can be decreased to provide an enclosure that is more suitable for human or animal implantation.

The piston 30 can also be used with existing osmotic delivery systems that utilize conventional pistons to increase the duration of continuous or pulsatile delivery of a benefi-

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cial agent from the osmotic delivery system. This is because the recess 34 can receive additional osmotic agent or because the existing osmotic agent can be located within the recess 34 so that the delivery system can hold additional beneficial agents. The factors that determine how much osmotic agent is needed to obtain sustained release of beneficial agents from osmotic delivery systems are described in a publication by F. Theeuwes and S. I. Yum, *Principles of the Design and Operation of Generic Osmotic Pumps for the Delivery of Semisolid or Liquid Drug Formulations*, ANNALS OF BIOMEDICAL ENGINEERING 41, 1976, at 343–353, the entire disclosure of which is hereby incorporated herein by reference.

In assembling the osmotic delivery system 20 according to one embodiment of the present invention, the sleeve 40 is first inserted into the recess 34 of the piston 30. Then, the piston 30 is inserted into the first opening 51 of the capsule 22. Once the osmotic agent pellet or tablet has been formed, it is placed inside the recess 34 such that the hollow interior 41 of the sleeve 40 receives the osmotic agent 26. If the osmotic agent is a powder formulation, it can be poured into the recess 34. After the osmotic agent is located within the capsule 22, the semipermeable body 28 is inserted into the first opening to close-off the first end 50 of the enclosure 21. At this stage of the assembly process, the osmotic agent 26 is located between the semipermeable body 28 and the piston body 32. The beneficial agent 24 is then inserted into the second opening 53 of the capsule 22 such that the beneficial agent is directly adjacent to the piston 30. Thereafter, the plug-like member 42 having means for sealing or ribs 48 extending outwardly from the outer surface thereof is inserted into the second opening to close-off the second end of the enclosure 21 and complete the osmotic delivery system 20.

FIG. 5 illustrates an alternative embodiment of an osmotic delivery system 120 according to the present invention. The foregoing and following discussion of the benefits and function of the osmotic delivery system 20 also applies to the osmotic delivery system 120. Thus, the osmotic delivery system illustrated in FIG. 4 has been assigned corresponding reference numbers as the osmotic delivery system 20, increased by 100. The osmotic delivery system 120 illustrated in FIG. 4 also includes many additional features and inherent functions as described further below.

As illustrated in FIG. 5, the osmotic delivery system 120 includes an elongated substantially cylindrical capsule 122 having an opening through which a semipermeable body 128 has been inserted. The semipermeable body 128 is a cup-shaped membrane that has been inserted into an opening in the first end 150 of the capsule 122.

Also located within the capsule 122 is the osmotic agent 126, which is a powder formulation. The osmotic agent 126 is received by the recess of the piston body 132, as is the sleeve 140. Because the osmotic agent 126 is a powder formulation, it generally occupies the entire space or gap between the piston body 132 and the semipermeable body 128. Thus, the powder formulation of the osmotic agent efficiently utilizes the space between the piston body 132 and the semipermeable body 128.

The capsule 122 of the osmotic delivery system 120 defines a delivery port 144 at the second end 152. Attached to the delivery port 144 is a catheter or tube 160 that delivers the beneficial agent dispensed from the capsule 122 to a remote location. Hence, the osmotic delivery system 120 does not include a plug-like member having a delivery port such as the plug-like member 42 shown in FIG. 1. Protru-

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sions or ribs 138 of the piston body 132 seal the beneficial agent 124 from the osmotic agent 126.

While the invention has been described in detail with reference to a preferred embodiment thereof, it will be apparent to one skilled in the art that various changes can be made, and equivalents can be employed without departing from the spirit and scope of the invention.

We claim:

1. An osmotic delivery system comprising:
an enclosure, said enclosure having an interior for holding
a beneficial agent, said interior having an interior
surface;

an osmotic agent located in said interior;
a semipermeable body in liquid communication with said
enclosure for permitting liquid to permeate through
said semipermeable body to said osmotic agent; and
a piston located within said interior of said enclosure,
being movable with respect to said interior of said
enclosure, defining a movable seal with said interior of
said enclosure that separates said osmotic agent from
said beneficial agent, and having a recess that receives
a sleeve having an interior that receives at least a
portion of said osmotic agent, said osmotic agent
located between said piston and said semipermeable
body, said osmotic agent for imbibing liquid from a
surrounding environment through said semipermeable
body to cause said piston to move and in turn cause
delivery of said beneficial agent from said enclosure,
wherein said osmotic agent consists of a semisolid or a
solid.

2. The osmotic delivery system according to claim 1,
wherein said osmotic agent is in the form of a tablet, pellet,
or powder at least partially within said recess.

3. The osmotic delivery system according to claim 1,
wherein said interior of said enclosure includes said interior
surface, said piston abutting against said interior surface.

4. The osmotic delivery system according to claim 1,
wherein said semipermeable body is located at least partially
within said interior of said enclosure.

5. The osmotic delivery system according to claim 1,
wherein said enclosure includes an opening and said semi-
permeable body includes a semipermeable plug, said semi-
permeable plug located at least partially within said opening.

6. The osmotic delivery system according to claim 1,
wherein said enclosure includes an opening and said semi-
permeable body includes a semipermeable membrane, said
semipermeable membrane located at least partially within
said opening.

7. The osmotic delivery system according to claim 1,
wherein said enclosure is a capsule.

8. The osmotic delivery system according to claim 1,
wherein said enclosure includes a cylindrical tube.

9. The osmotic delivery system according to claim 1,
wherein said osmotic agent is located between said semi-
permeable body and said piston.

10. The osmotic delivery system according to claim 1,
wherein a portion of said osmotic agent is located outside of
said recess.

11. The osmotic delivery system according to claim 1,
wherein said piston is cup-shaped.

12. The osmotic delivery system according to claim 1,
wherein said recess is cylindrical.

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13. The osmotic delivery system according to claim 1,
wherein said recess is a square cavity, concave indentation,
conical pit, gouge, or depression.

14. The osmotic delivery system according to claim 1,
wherein said piston includes at least one rib for effecting a
movable seal with said enclosure.

15. The osmotic delivery system according to claim 1,
wherein said piston includes a plurality of ribs for effecting
said movable seal with said interior.

16. The osmotic delivery system according to claim 1,
wherein said sleeve is cup-shaped.

17. The osmotic delivery system according to claim 1,
wherein said beneficial agent is located in said interior of
said enclosure, said beneficial agent being delivered from
said enclosure when said piston moves.

18. The osmotic delivery system according to claim 1,
wherein said beneficial agent is located in said interior of
said enclosure, said piston defining said movable seal that
separates said osmotic agent from said beneficial agent.

19. The osmotic delivery system according to claim 1,
further comprising: an opening in said enclosure; and a plug
located in said opening, said plug having a delivery port for
delivery of said beneficial agent from the interior of said
capsule.

20. The delivery system according to claim 1, further
comprising: a delivery port in said enclosure for delivery of
said beneficial agent from the interior of a capsule.

21. The osmotic delivery system according to claim 1,
wherein said osmotic agent comprises an osmagent or an
osmopolymer.

22. The osmotic delivery system according to claim 1,
wherein said osmotic agent is magnesium sulfate, magne-
sium chloride, potassium sulfate, sodium chloride, sodium
sulfate, lithium sulfate, sodium phosphate, potassium
phosphate, d-mannitol, sorbitol, inositol, urea, magnesium
succinate, tartaric acid, raffinose, sucrose, glucose, lactose,
fructose, dextran, poly(hydroxy-alkyl methacrylates) with
molecular weight of about 30,000 to about 5,000,000, poly
(vinylpyrrolidone) with molecular weight of about 10,000 to
about 360,000, anionic and cationic hydrogels, polyelectro-
lyte complexes, poly(vinyl alcohol) having low acetate
residual, optionally cross-linked with glyoxal, formaldehyde
or glutaraldehyde and having a degree of polymerization of
about 200 to about 30,000, a mixture of methyl cellulose,
cross-linked agar and carboxymethylcellulose, a mixture of
hydroxypropyl methylcellulose and sodium
carboxymethylcellulose, a polymer of N-vinylactams,
polyoxyethylene-polyoxypropylene gels, a
polyoxybutylene-polyethylene block copolymer gel, carob
gum, polyacrylic gel, polyester gel, polyurea gel, polyether
gel, polyamide gel, polypeptide gel, polyamino acid gel,
polycellulosic gel, carbopol acidic carboxy polymer having
a molecular weight of about 250,000 to about 4,000,000,
Cyanamer polyacrylamide, cross-linked indene-maleic
anhydride polymer, polyacrylic acid having a molecular
weight of about 80,000 to about 200,000, Polyox Polyeth-
ylene oxide polymer having a molecular weight of about
100,000 to about 5,000,000, starch graft copolymer, or an
acrylate polymer polysaccharide.

* * * * *



US005108927A

United States Patent [19]
Dorn

[11] **Patent Number:** **5,108,927**
[45] **Date of Patent:** * Apr. 28, 1992

[54] **SPECIMEN TRANSPORT DEVICE
CONTAINING A SPECIMEN STABILIZING
COMPOSITION**

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[73] Assignee: **Wadley Technologies, Inc., Dallas,
Tex.**

[*] Notice: The portion of the term of this patent
subsequent to Dec. 3, 2008 has been
disclaimed.

[21] Appl. No.: **269,490**

[22] Filed: **Nov. 9, 1988**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 772,954, Sep. 4, 1985,
abandoned, which is a continuation-in-part of Ser. No.
525,164, Aug. 23, 1983, abandoned, which is a con-
tinuation-in-part of Ser. No. 431,776, Sep. 30, 1982,
abandoned.

[51] Int. Cl.⁵ **C12M 1/24; C12N 1/00;**
B65D 81/00

[52] U.S. Cl. **435/296; 435/299;**
435/243; 128/760; 128/765

[58] Field of Search 435/4, 29, 34, 296,
435/286, 284, 243, 299; 128/760, 764, 765;
604/214, 220

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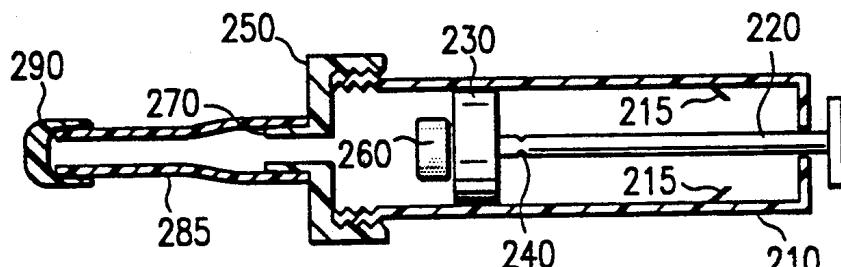
[57] **ABSTRACT**

The improvement of specimen quality for microbial analysis is addressed by the present invention which discloses a chemical composition for use in a method and apparatus for transporting a specimen suspected to contain microorganisms of interest to a laboratory for analysis and improved methods of analysis.

A device and method for taking, storing, and preserving fluid samples is disclosed comprising a container and a composition designed to maintain the level of microorganisms present in a specimen during transportation of the specimen to a testing facility.

The method and apparatus can be utilized on all types of aqueous specimens and specimens which may be extracted in aqueous solution for analysis of microorganisms therein.

14 Claims, 6 Drawing Sheets



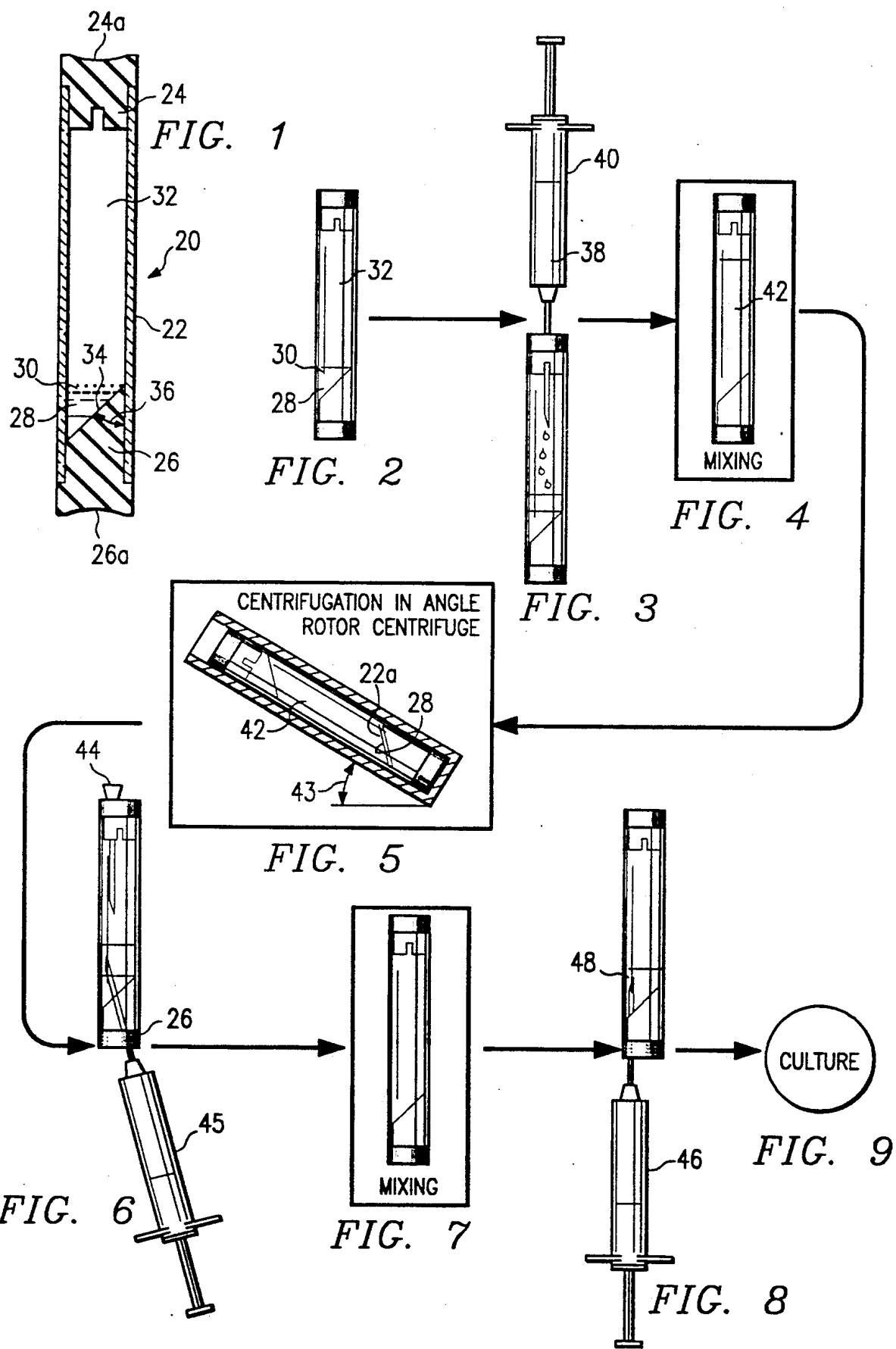
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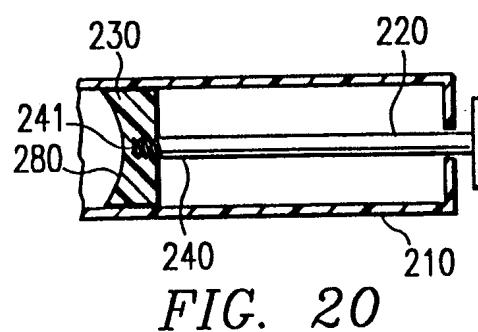
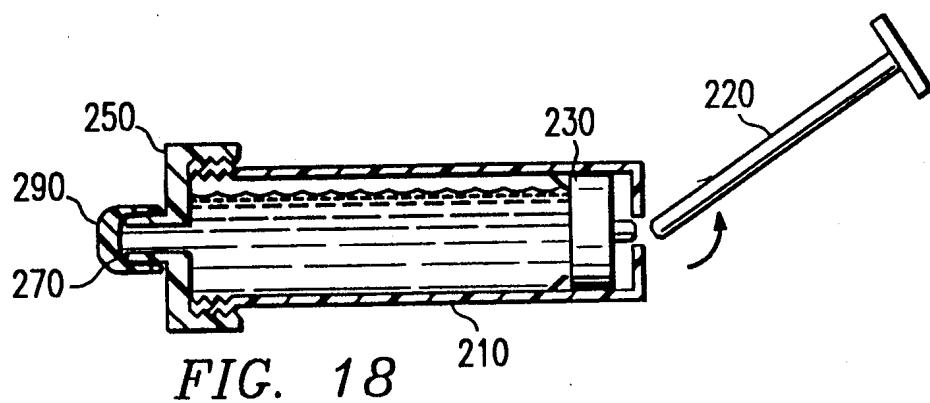
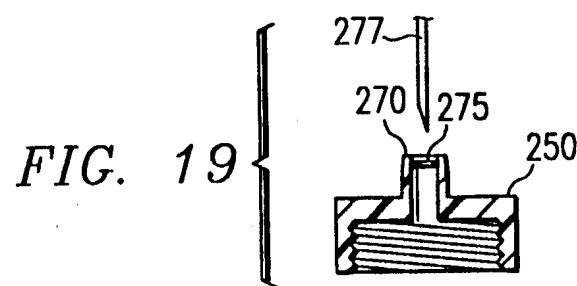
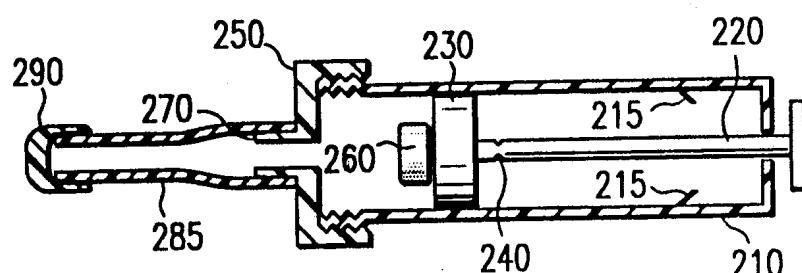
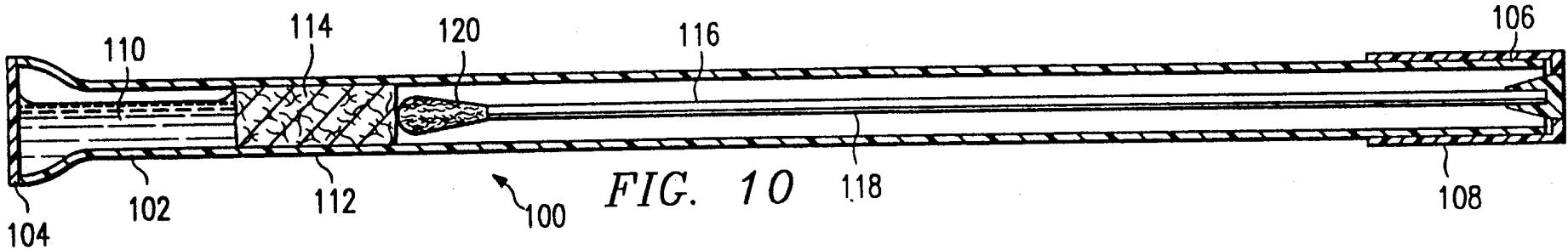
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SURVIVAL VALUES OF AN ORGANISM IN THE PRESENCE
OF ANTIBIOTICS USING CURRENT TRANSPORT DEVICES
(5 MINUTE INTERVALS)

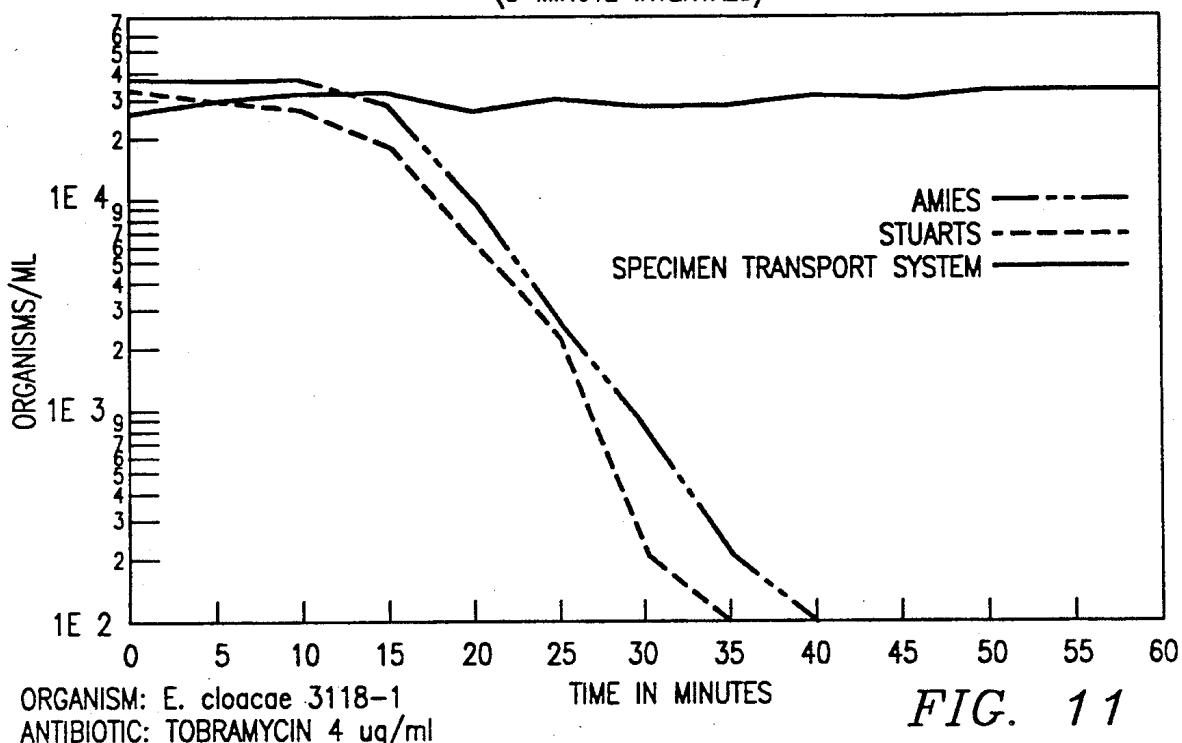


FIG. 11

SURVIVAL VALUES OF AN ORGANISM IN THE PRESENCE
OF ANTIBIOTICS USING CURRENT TRANSPORT DEVICES

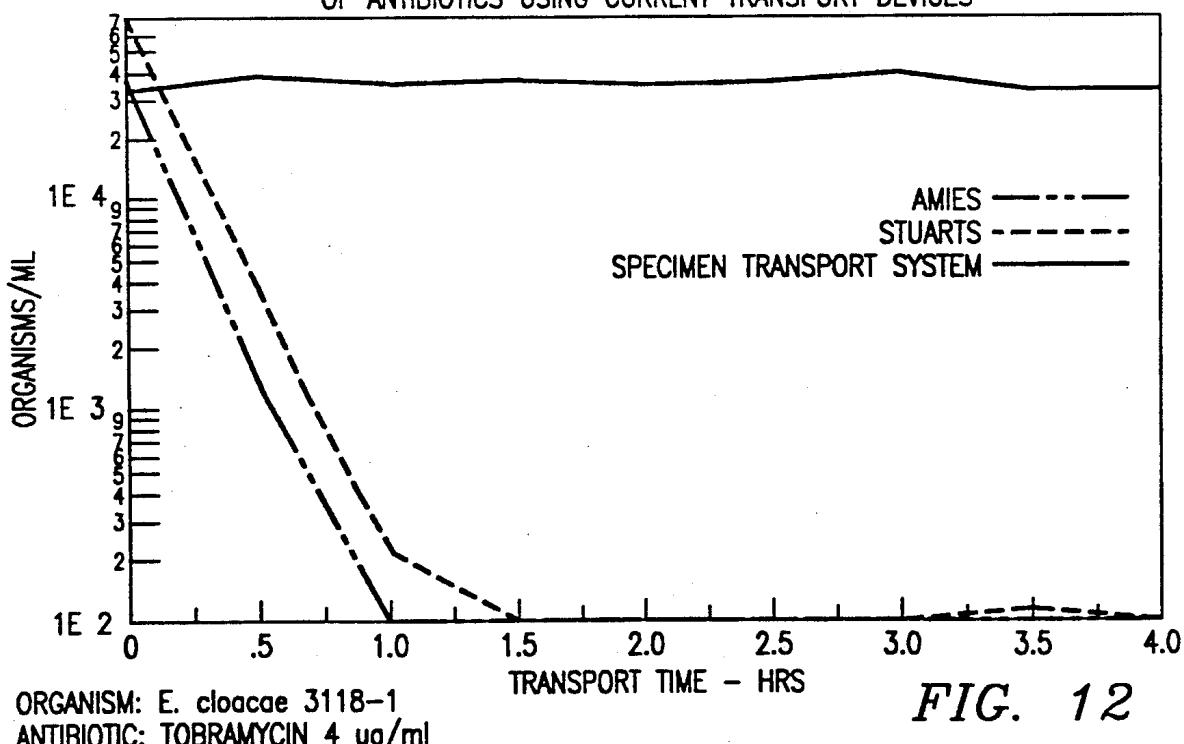


FIG. 12

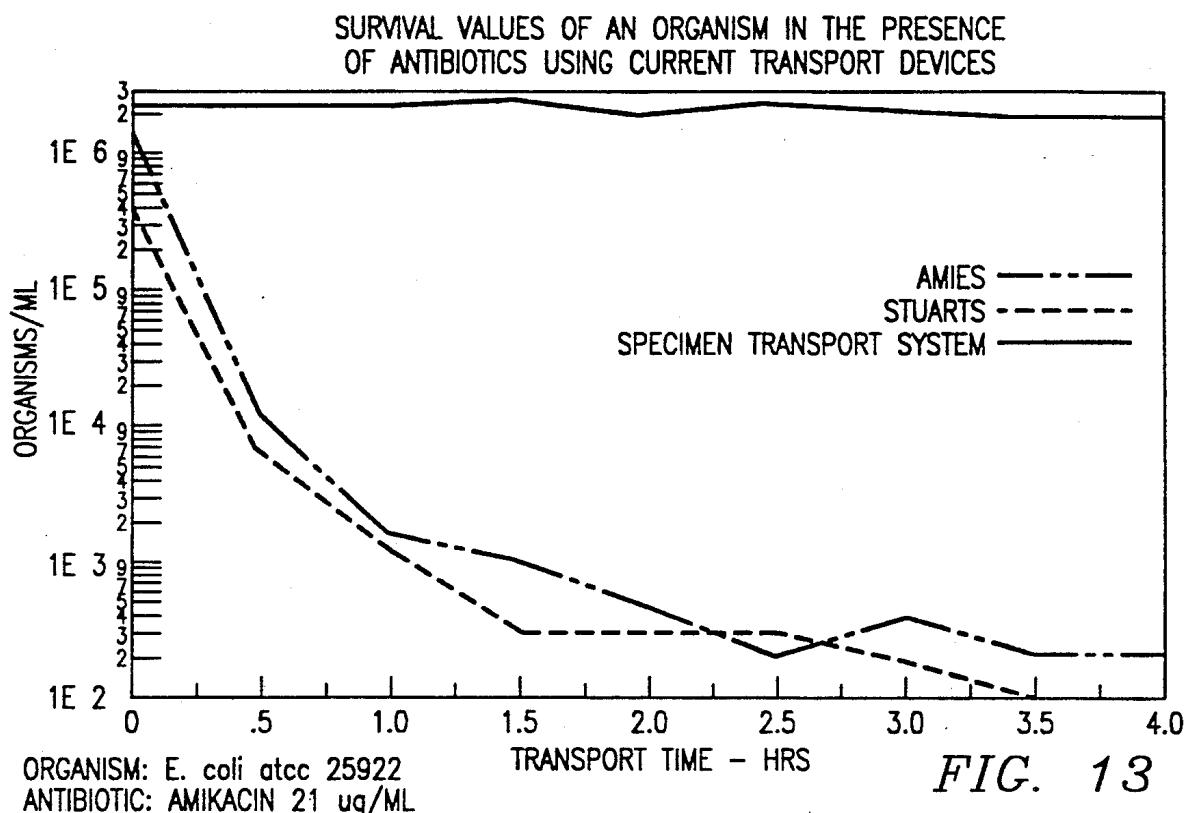


FIG. 13

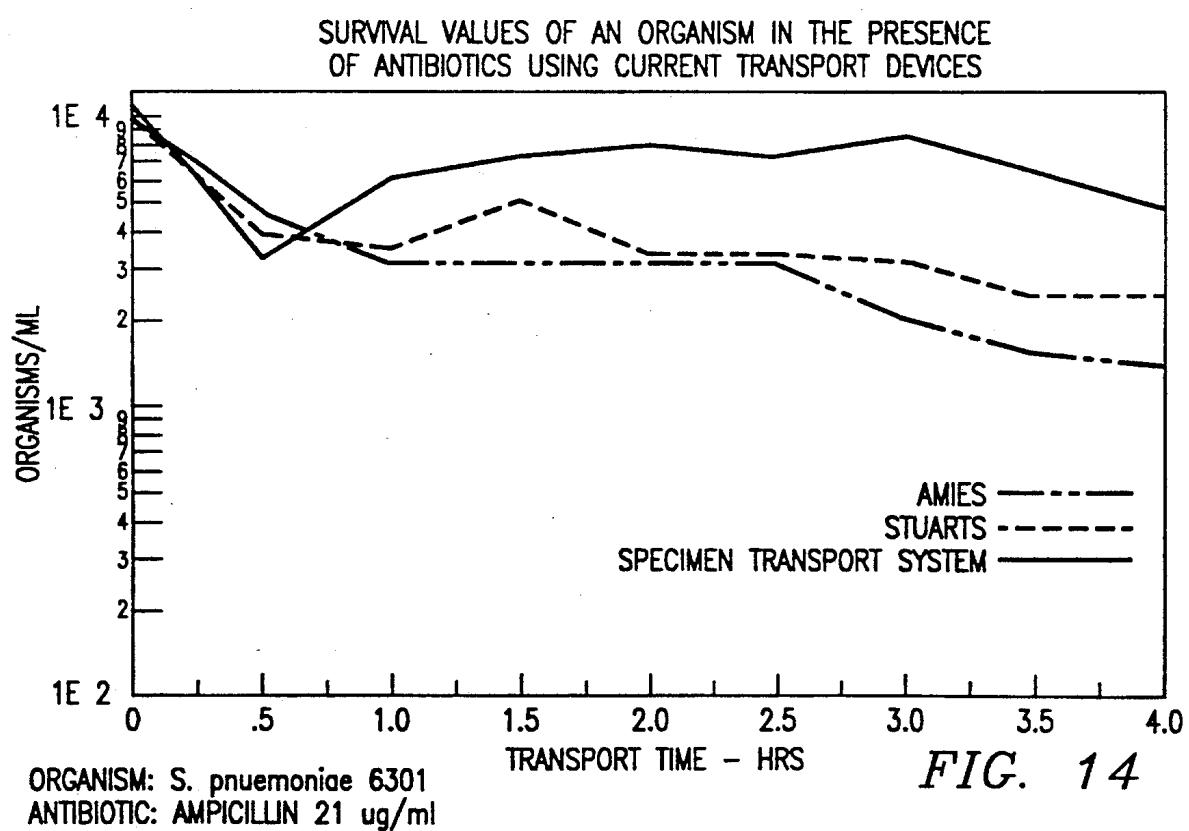


FIG. 14

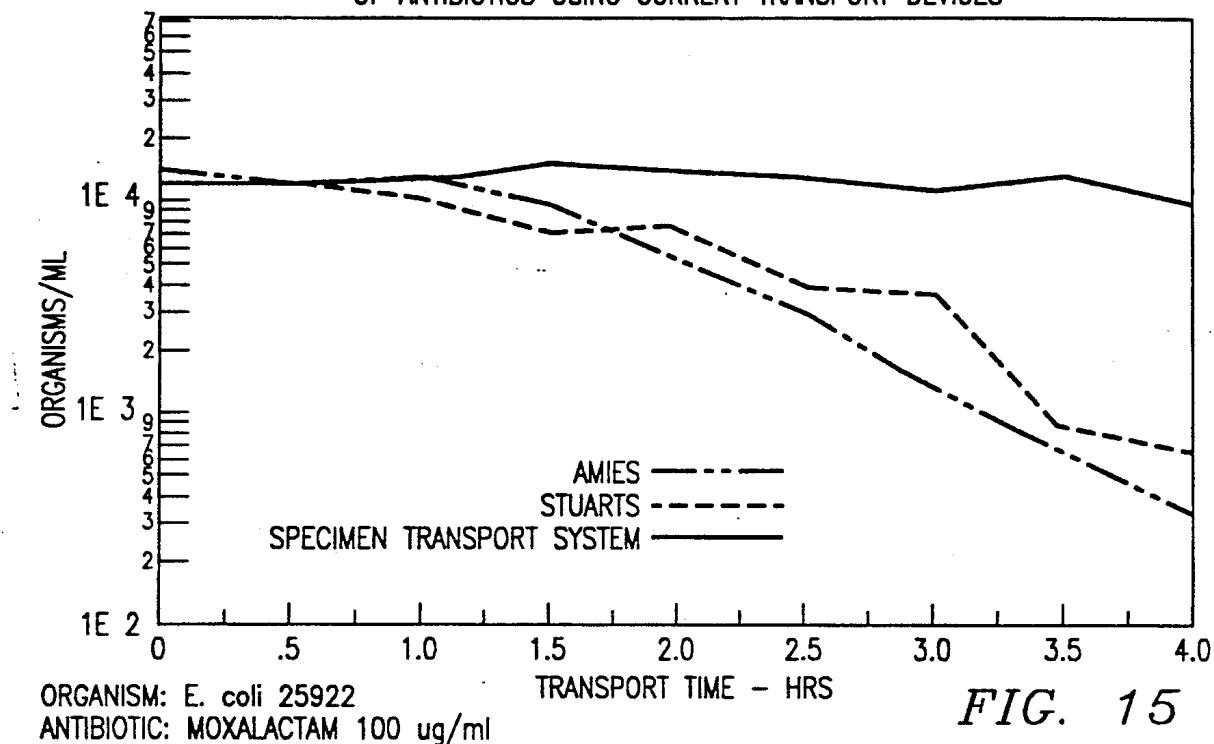
SURVIVAL VALUES OF AN ORGANISM IN THE PRESENCE
OF ANTIBIOTICS USING CURRENT TRANSPORT DEVICES

FIG. 15

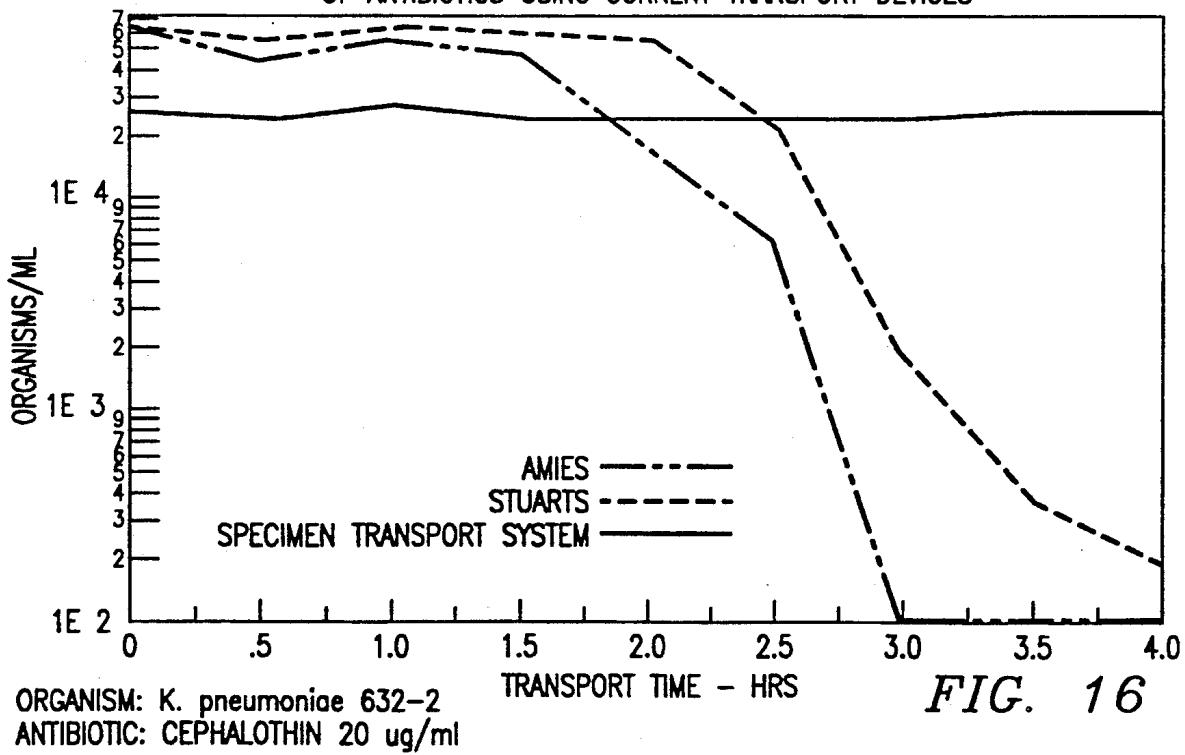
SURVIVAL VALUES OF AN ORGANISM IN THE PRESENCE
OF ANTIBIOTICS USING CURRENT TRANSPORT DEVICES

FIG. 16

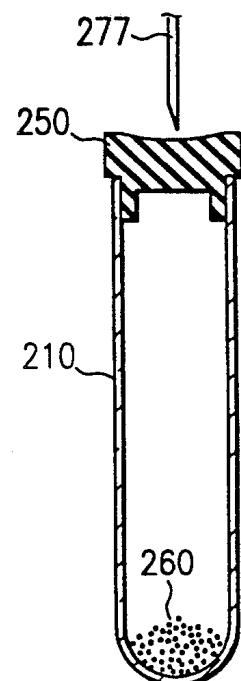
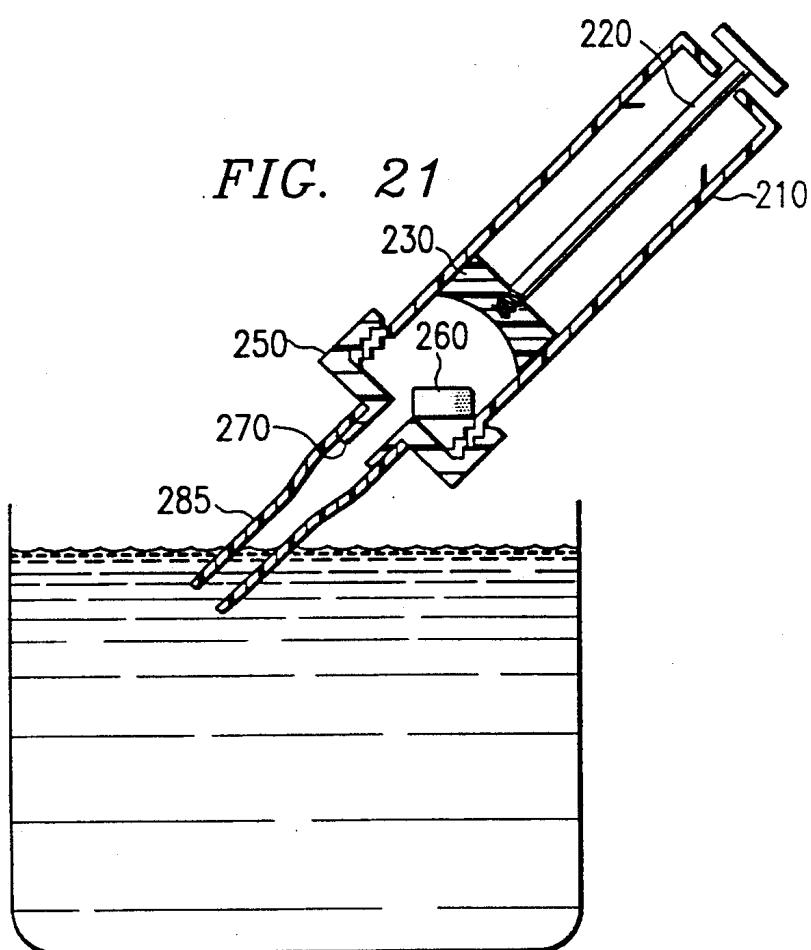


FIG. 23

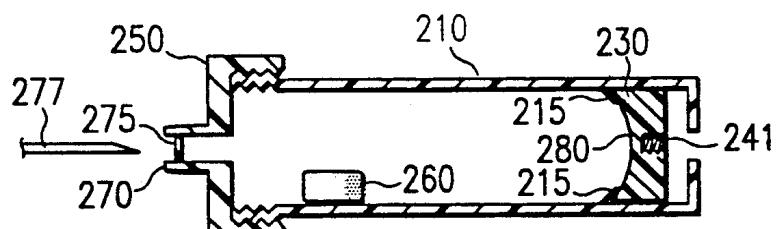


FIG. 22

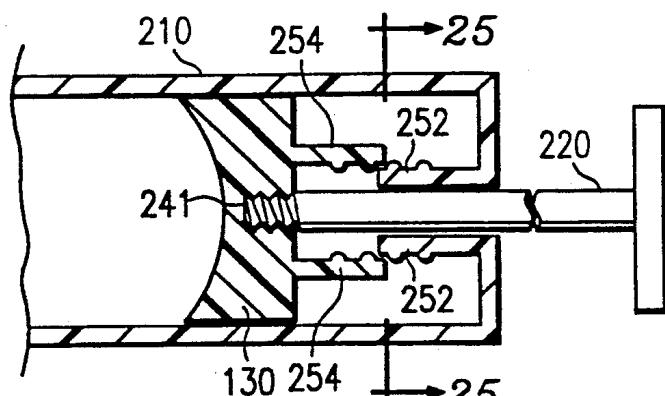


FIG. 24

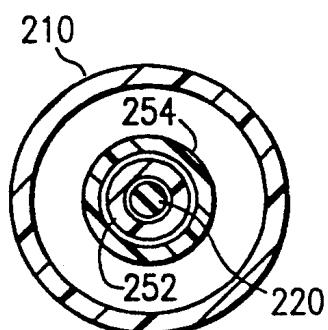


FIG. 25

SPECIMEN TRANSPORT DEVICE CONTAINING A SPECIMEN STABILIZING COMPOSITION

This is a continuation-in-part of copending application Ser. No. 06/772954 filed Sep. 4, 1985, now abandoned, which is a continuation-in-part of copending application Ser. No. 06/525164 filed Aug. 23, 1983, now abandoned, which is a continuation-in-part of copending application Ser. No. 06/431776, filed Sep. 30, 1982, now abandoned.

TECHNICAL FIELD

This invention relates to the field of analysis of microorganisms in a specimen. In particular, this invention relates to maintaining the quality or microbial integrity of a specimen from the time of collection to the time laboratory analysis is initiated.

BACKGROUND ART

Accurate laboratory analysis of specimens suspected of containing microorganisms is of utmost importance in the fields of medicine and food technology and safety, among others. While techniques have been developed for improving the rapidity and sensitivity of microbiological identification, drugs have been developed for fighting infection in patients, and sanitary conditions for food processing have become mandated by law, it is evident that problems remain.

For example, septicemia, which is the presence of pathogenic microorganisms in the blood, is one of the most serious types of infections encountered. There is unanimous agreement in the medical profession that septicemia is second only to meningitis in terms of serious infections. Even though modern medicine has provided an armament of antibiotics and antifungal drugs, the mortality rate from septicemia is approximately twenty-five percent. Also, when shock accompanies septicemia, the mortality rate increases to over sixty percent. Debilitating diseases, major surgery, administration of immunosuppressive drugs or anticancer medications cause patients to be particularly prone to septicemia. Early diagnosis of the causative agent in conjunction with the use of the appropriate antibiotic therapy is essential in fighting septicemia. Consequently, it is imperative that the physician know as rapidly as possible, not only that the patient has septicemia, but also the identity and/or antibiotic susceptibility of the microorganisms involved. Thus, proper and timely diagnosis of septicemia depends upon very rapid and efficient analysis of the microorganisms in patient's blood. Further, it is necessary during the analysis of the microorganisms in the patient's blood that the blood sample not be contaminated with microorganisms from the hospital environment.

Another example of a disorder caused by microorganisms is the presence of pathogenic microorganisms in the urine, which occurs most commonly in infants, pregnant women, patients with obstructive lesions, following the use of instrumentation in the urinary tract (such as catheters), or with urologic diseases affecting micturition. This disorder can result in a localized infection within the bladder or kidneys. When confined to the bladder, the infection is usually well controlled by antimicrobial therapy. Once the kidneys are infected, however, lesions may continue to progress despite treatment leading to chronic pyelonephritis and septicemia.

In the field of food technology, contamination occasionally becomes a problem that endangers human health. Contamination of milk, for example, has been known to occur even where a processing step to kill harmful microorganisms is employed because equipment malfunctions, human error, and sometimes mysterious circumstances contribute to processing ineffectiveness. In such cases, rapid and accurate analysis of specimens from the food processing apparatus and the food itself are important in establishing the cause of the contamination so that the process may be remedied. Various techniques are utilized for analysis of microorganisms. Simple quantitative analysis involves determining the number of microorganisms in a given specimen regardless of microorganism identity. Quantitation may be accomplished by introducing a known volume of specimen (perhaps diluted by a known amount in a nutrient broth) onto a nutrient agar and allowing formation of colonies. It may be desirable to determine the identity and/or antibiotic susceptibility of the microorganisms found. Analysis to establish microorganism identity and/or susceptibility is usually accomplished by subjecting individual colonies to differentiating media.

In some instances, accurate quantitation as well as identification of particular microorganisms, rather than mere determination whether that particular microorganism is or is not present is highly important. Thus a determination that a specimen is "positive" for microorganisms or "negative" for microorganisms may be insufficient. Rather if the specimen is positive, it may be necessary to know how many microorganisms of a particular species are present in the specimen. It is normal for certain microorganisms to be present in the human mouth and throat at all times, for example. These normal microorganisms, referred to as normal flora, do not generally cause disease in the numbers normally present. However, it is possible for an organism that may be part of the normal flora to proliferate to such an extent that it becomes a disease-causing organism (pathogen). It can be discerned, therefore, that the difference between the normal state of a human throat, for example, and a diseased human throat may be not in the identity of a particular organism that may survive to the time of analysis, but in the numbers of that organism present in the patient's throat. Generally, the bloodstream is sterile. However, transient bacteremia may occur where a few organisms enter the bloodstream through a cut or sore, for example, which is not usually a cause for alarm. Quantitation of microbes in a blood specimen is highly important to distinguish transient bacteremia from septicemia and, perhaps, specimen contamination. While quantitation is of utmost importance in analyzing blood specimens, determining the identity of the microbial pathogen present is also important. Although it may not be necessary to identify a microorganism taxonomically to treat a patient, it may be important to determine microorganism susceptibility to antibiotics so that proper drug therapy may be chosen. This may be done by identifying the organism by genus and species since drug manufacturers often have pre-determined the effectiveness of a drug on particular taxonomic groups. Alternately, testing for drug effect (antibiotic susceptibility) may be accomplished.

In some fluids, microorganism concentration may be so low in the specimen that using conventional methods a tested portion will not reveal microbial presence.

Recently, improvements useful for detecting low concentrations of microorganisms have been disclosed which have greatly improved detection of septicemia in blood before microorganisms have proliferated to such an extent that the patient is in a severe disease state.

Recently developed method and apparatus for concentrating and detecting microorganisms from a sample fluid are disclosed in U.S. Pat. No. 4,131,512 entitled "Method of Detecting Microbial Pathogens Employing a Cushioning Agent" and its division, U.S. Pat. No. 4,212,948 entitled "Apparatus For Detecting Microbial Pathogens Employing A Cushioning Agent". The technique disclosed in the above patents involves (when analyzing a blood sample) pre-lysis of corpuscular compounds followed by centrifugation to concentrate the microorganisms away from the other constituents including antimicrobial factors present in the blood. The concentrated microorganisms are then placed upon a nutrient media such that substances inhibitory to microbial growth present in the sample is diluted a minimum of sixty-fold. It has been previously documented that this technique yielded more positive cultures than the conventional liquid broth culture, the pour plate method, or the filtration method using the solid matrix filter. Gordon Dorn, Geoffrey A. Land, and George E. Wilson, "Improved Blood Culture Technique Based on Centrifugation: Clinical Evaluation," 9 J. Clinical Microbiology 391-396 (1979).

A problem remains in the field of microbial analysis despite the increasing sophistication in techniques for detecting and determining the identity of microorganisms within a specimen because the accuracy of the techniques is limited by the microbial integrity of the sample analyzed. By "microbial integrity" it is meant that a specimen taken at one point in time (t_0) and analyzed at another point in time (t_1) will provide an accurate representation of the microbial population of interest in the patient, food supply or other source from which the specimen was taken, when the specimen is analyzed.

At least three major factors exist which contribute to the lack of microbial integrity of specimens at t_1 . The first is that specimens often contain antimicrobial factors which may kill microorganisms of interest before t_1 . A second factor is microorganisms of interest may not survive in the specimen until t_1 even if no antimicrobial factors are present. Third, certain microorganisms may reproduce much more rapidly in a specimen than, for example, in the patient from whom the specimen was taken. Fast-growing but relatively harmless or irrelevant microorganisms may overwhelm the specimen so that more harmful species of interest are not detected by the analyzing laboratory. Failure to detect the important organism causes misinterpretation of the contamination problem even though the laboratory may correctly identify the organisms that have proliferated. In each case, the sample analyzed at t_1 will not give an accurate picture of the microbial problem in the patient or other source. Since drug therapy prescribed by a physician may be dependent on laboratory determinations of type of infecting microorganisms and degree of infection, solving the problem of microbial integrity may be vital to the recovery of the patient. False negatives with respect to food processing equipment or food itself may be detrimental to public health. In addition, misidentification of contamination in the food-related area may prevent discovering the source of contamination or cause the needless disposal of products. Discov-

ering the source is often necessary to prevent future incidents of contamination.

Where antimicrobial factors, such as antibiotic drugs, are present in a specimen several problems arise. For example, a patient given antibiotics by his or her physician may have a level of such drugs in the blood or urine. At t_0 , when a urine specimen is taken (for example), the urine may contain living microorganisms and some antibiotic. The antibiotic may continue to work to kill the microorganisms in the specimen so that at t_1 , no living microorganisms remain. The laboratory may test the urine specimen and conclude that the patient no longer has a microbial problem. However, this may be inaccurate. Unlike the specimen, the patient's system may continue to be seeded with microorganisms from the source of infection. While the level of antibiotics in the specimen might be sufficient to kill microorganisms therein, this does not necessarily reflect the status of the infection within the patient. Additionally, living organisms are required for identification and antibiotic susceptibility testing of microorganisms. If the specimen arriving at the laboratory has no living microorganisms, the laboratory cannot usually accurately identify the organisms nor determine antibiotic susceptibility. Drugs which may be more effective in eliminating particular organisms may not be prescribed if a less effective drug is taken by a patient and is effective enough to destroy the microbial integrity of the specimen taken from that patient, even though it is not effective enough in the patient's system to destroy the infecting microorganisms. Natural bacteriocidal substances found in some specimens, such as blood, may also change the microbial integrity of the specimen before it is analyzed causing inaccurate results.

Even if no antimicrobial factors are present in a specimen, a microbial integrity problem remains. If living microorganisms are contained in a specimen at t_0 , but fail to survive to t_1 , no microorganisms will likely be detected by the laboratory because detection techniques are chiefly based on microorganism reproduction. Such a situation will lead to false negative reports and potentially harmful consequences if microbial infections or contaminations go untreated.

Organisms may reproduce so well in a specimen that laboratory analysis will falsely indicate that the patient, foodstuff, or food processing equipment is highly contaminated. Incorrect drug therapy may be administered that is both unnecessary and potentially harmful by itself to some patients. Also, the rapidly-reproducing organism may cause other more harmful microorganisms in the specimen to die in the specimen, although they may be reproducing rapidly in the patient. Since appropriate drug therapy may differ depending on the identity of the problem organism, the patient may not be treated properly for eliminating the more virulent, undetected microorganism and will thus be harmed. In the case of food analysis, misidentification of the source of contamination may result and thus the source which introduced the virulent microorganism may not be discovered.

The problem of lack of microbial integrity in specimens may be increased because of hospital inefficiency in transporting the specimen to the laboratory and backlogs occurring in the laboratory of samples to be analyzed. Although most textbooks and handbooks of microbiological technique mandate a specimen hold time of less than two hours, it is often impractical to comply with this standard of efficiency. The problem may be

even worse when the specimen must be transported from a remote site such as a doctor's office, a food processing plant, or a sewage-treatment plant to a central laboratory. The accuracy of analysis decreases the longer it takes to transport the specimen to the laboratory because of the deterioration of microbial integrity of the specimen.

Thus, ideally, when testing fluids for differing levels of organisms, the level of organisms present in the sample should not change between the time a sample is taken, and the time the sample is tested. However, the environment of a test sample is usually different from the environment of the tested fluid due to varying temperature, light, availability of nutrients, etc. This can cause large differences in the number of organisms present at the time of testing. For example, the number of live bacteria in a urine sample which has been out of the donor's body for a few hours can increase due to reproduction, or they can decrease due to the action of antibiotics which the donor may be taking. Likewise, organisms present in water can quickly consume all available nutrients in a sample container and die before a test can be conducted. In any case, the longer the time between taking the sample and testing the sample, the higher probability of an incorrect test result there will be.

While the specimen quality problem has been addressed by the art, no known approach has been entirely effective and some have introduced further problems.

The simplest approach disclosed by the prior art is rapid transfer from the point of specimen collection to the point of analysis. For organisms particularly sensitive to transport, immediate streaking on nutrient plates has been suggested literally at the bedside of the patient. As pointed out, it is often difficult to make sure that a specimen has been transported within a recommended time frame. Even if it has, if the specimen contains antibiotics, up to 50% of the microorganisms of interest may be killed within 15-20 minutes. Thus, it can be seen that transport to a lab in two hours or less may be insufficient. Immediate streaking at bedside may cause loss of aseptic technique and the remaining problem of transport of the plate to the laboratory. Antibiotic presence may still present a problem.

The transport of specimens in the past has often been undertaken in initially sterile containers in an attempt to improve specimen quality. Even if a specimen is collected in a sterile container, however, the microbial integrity of the specimen may deteriorate during transport because initial container sterility neither prevents death nor overgrowth of microbes in the specimen. Additionally, sterility of containers could be lost where such specimens as urine, for example, are collected as soon as the closure means is removed for micturition.

In U.S. Pat. No. 4,145,304 ('304) and U.S. Pat. No. 4,174,277 ('277), a method and structures for the removal of antimicrobial factors were disclosed. A mixed resin bed adsorbs the antibiotics to prevent cidal effects on the microorganisms of interest. Multiple physical entries into the specimen are required in the resin bed system in that the specimen must be collected from the patient, transferred to the resin bed for adsorption of antibiotics, and removed from the resin bed. The more physical entries a specimen is subjected to, the higher the risk of microbial contamination from the skin of the operator or the environment. The resin bed is insoluble and therefore requires physical manipulations before the specimen may be analyzed. Loss of microorganisms may result from some non-selective adsorption. Addi-

tionally, the mixed resin system fails to address the maintenance of microbial cells in a viable condition without replication.

Certain systems are taught for use in urine specimens which address the problem of uncontrolled growth of particular species of interest which could skew analysis. However, most of these systems focus on killing bacteria that may be present since the specimen will be assayed for general chemical levels, such as glucose, bilirubin etc. In systems taught for preserving microbial integrity, antibiotic blockage is generally not addressed. Thus, no means of preserving the actual count of microorganisms in the presence or absence of bactericidal agents is addressed by known urine specimen-treating agents.

Maintaining a specimen at about 4° C. from the time of collection to the time of analysis is another known approach to attempting to maintain specimen quality. Since low temperature may slow microbial growth, antibiotics which act on only replicating organisms may lose effectiveness. However, this approach is impractical in the field, and the low temperature may detrimentally affect the viability of certain microorganisms while being an ineffective control on the growth of others. Additionally, the action of antibiotics is not necessarily controlled by the low-temperature approach. An example of a microorganism which may be killed by the cold is *Streptococcus pneumoniae*, one that a physician would be interested in detecting as it is an etiological agent of lobar pneumonia disease. Thus, it is preferable to maintain the sample at room temperature of about 21-25° C.

Other methods for improving specimen quality include Amies (C. Amies and F. Path, 58 *Canadian J. Public Health* 296 (1967)) and Stuarts (R. Stuart et. al., "The Problem of Transport of Specimens For Culture of Gonococci," 45 *Canadian J. Public Health* 73 (1954)). These methods may provide some improvement of specimen quality for some microorganisms of interest, however these systems fail to address the possible presence of antibiotics in a specimen, the differing nutritional needs of different microorganisms, and the effect of specimen hold time on accurate microorganism quantitation.

Another problem left unaddressed by previous approaches to microbial detection is the possibility that additional microorganisms will be introduced to a specimen from an external source. This "contamination" of the specimen will cause inaccurate results since, for example, a patient may be deemed to have a microorganism in the blood that in fact is not present. Contamination of specimens becomes more likely the more the specimen is transferred from container to container and the more it undergoes physical manipulations. For example, a commercially available system for urine specimen transport (Becton-Dickenson) requires manipulation from the urine collection vessel to the container with the preservative therein. It is therefore desirable to provide collection vessels which reduce the manipulations required, provide a means to instantly preserve the microbial integrity of a sample, and in a most preferred embodiment can be utilized for other processing steps in the analysis of microorganisms of interest.

Therefore, a method and means is needed for receiving a fluid sample suspected of containing microbial pathogens and antimicrobial factors which will minimize the risk of contamination, reduce or eliminate the requirement of sterility of the collection vessel for some specimens, provide for deactivation of antimicrobial

factors during the time that the sample is transported so that once the sample is removed from the collection and/or processing vessel and placed on growth media, the microorganisms of interest present in the sample including the fastidious microorganisms of interest will proliferate and become identifiable, and which will maintain the viability of at least some of the microorganisms of interest, preferably so that the microbial integrity of the sample is maintained from time of specimen collection (t_0) to the time of specimen analysis (t_1). 10

It has now been found that microbial integrity of patient specimens and other specimens may be preserved so that analysis at a t_1 up to about 72 hours after t_0 will result in a much more accurate representation of the microbial population in that sample than has previously been possible. This has been done by providing an admixture of individual chemicals which solubilize in an aqueous specimen to form a unique mixture which acts synergistically as a preservative of microbial integrity of the specimen. By "preservative" it is meant that the unique mixture prevents replication of microorganisms of interest, allows improved survival of said microorganisms until the inception of laboratory analysis, and blocks the action of antimicrobial factors that may be present in the specimen. By "microorganisms of interest" it is meant the microorganisms to be tested for in the laboratory protocol. It may not be necessary or desirable to preserve the viability, for example, of every possible microorganism that may be present in a given specimen. In the food industry, for example, non-harmful or even beneficial microorganisms may be present in food which a laboratory would not be interested in identifying. However, the laboratory would be interested in testing for microorganisms potentially harmful to human health. Therefore, preservation of the latter "microorganisms of interest" would be addressed by the present invention. In addition, the growth of the microorganisms which are not of interest must be kept in check to prevent masking of the harmful microorganism in the analysis procedure, and to prevent the rapidly producing non-harmful organisms from depleting the nutrients and causing death of other microbes. The present invention is effective in inhibiting replication of such potentially interfering organisms. The present invention thus allows a longer time to elapse between 45 specimen collection and specimen analysis than has previously been possible without sacrificing accuracy. It also allows for more accurate analysis even if a sample is analyzed within a short time period because it blocks the action of antimicrobial factors which may 50 destroy microorganisms of interest even within the two hour processing time period recommended in the prior art.

In addition, no reason is known why the disclosed specimen transport system would not be advantageous for improving the accuracy of analysis of specimens for periods exceeding 72 hours. If the viability of even a few microorganisms of interest is maintained, the microbial integrity of specimen analyzed will be improved over that possible according to the prior art, resulting in 60 improved laboratory analysis.

Disclosed is a novel method, article and compositions for detecting microbial pathogens. In another aspect, this invention relates to a novel technique and means for selectively separating microorganisms from a sample 65 fluid which contain antimicrobial factors. In still another aspect, this invention relates to a method and means for use in the detection of microbial pathogens

which provides improved recovery of microorganisms. In yet another aspect, this invention relates to a method and means for accurately quantitating the number of microorganisms present in a sample fluid at a given time when quantitated at a later time.

An article for receiving specimens is disclosed which includes a means for preserving the microbial integrity of the specimen.

SUMMARY OF THE INVENTION

According to the invention, there is provided an apparatus for stabilizing the level of microorganisms in a sample comprising a container and a composition effective for preserving the microbial integrity of the specimen. The composition is located in the container whose structure provides for admixing of the specimen and the composition as the specimen enters the container.

Also according to the invention, there is provided a method for collection and transportation of fluid specimens comprising the admixing of a composition effective for preserving the microbial integrity of the specimen with the specimen in a container; the container's structure providing for the admixing of the specimen and the composition as the specimen enters the container.

Further, in accordance with the invention, compositions and methods for deactivating antimicrobial factors and maintaining the microbial integrity within a specimen after it has been collected and before the microorganisms of interest are analyzed are also disclosed.

According to a preferred embodiment of the invention, admixing of the composition and specimen takes place in a container comprising a screw on cap (thereby reducing the chances of contamination of the test personnel or the specimen when the cap is removed and replaced as may occur when a rubber stopper is used), a slidable piston within the container, and a detachable piston rod wherein the rod may be removed from the piston (leaving no portion of the rod outside of the container and allowing the container to be placed in a test tube rack, stood on a table, or mounted in a centrifugation device).

Also, according to a preferred embodiment of the subject invention, a particular class of compositions, effective for preserving the microbial integrity of the specimen, (hereafter, the specimen transport system) soluble in aqueous solution effective for deactivating antimicrobial factors within a specimen containing said antimicrobial factors and microorganisms and method of use thereof, is provided which serves the following purposes:

(1) immediate blockage of the cidal action of penicillins, cephalosporins, and aminoglycosides, and antibiotics which require microbial growth for effectiveness;

(2) initiation of anaerobic conditions to allow maintenance of the life of fastidious organisms susceptible to the lethal action of oxygen;

(3) complete neutralization of the cidal action of normal human blood and cidal components inherent in other specimens;

(4) to hold stable the viable count of microorganisms over a period of time; and

(5) provide for the optimal nutritional needs of the microorganisms of interest.

The procedure can be utilized on all types of body fluids such as blood, bone marrow, spinal and pleural fluids, body secretions, urine and the like as well as

non-fluid specimens from a patient from which microorganisms may be extracted in aqueous solution. The microbial integrity of water supply specimens, food specimens and samples of surface contamination of food preparation or processing equipment and other specimens are also appropriately preserved with the present invention. Generally, when employed in connection with a blood sample, a lysing agent will be employed. A mucolytic agent may be advantageously employed with sputum. An example of an effective lysing or mucolytic agent is detoxified saponin which is disclosed in U.S. Pat. No. 4,053,363 to Dorn, et al. The novel composition of the subject invention can be utilized in a sample collection or transporting container and allowed to be admixed with the sample after it has been collected but before microbial pathogens therein are analyzed by a method such as, for example, depositing them upon a growth media for microbial pathogens. The novel composition of the subject invention can be in the form of an aqueous solution contained within said sample collection and transporting container. However, the novel composition for specimen transport is preferably positioned in said container in the form of solid particles which are soluble in the sample fluid or the aqueous extract of the specimen as the case may be.

It is envisioned that the subject invention can be utilized within the lysis-centrifugation devices such as disclosed in U.S. Pat. No. 4,212,948 issued Jul. 15, 1980 and entitled "Apparatus For Detecting Microbial Pathogens Employing A Cushioning Agent", which employs the basic method disclosed in U.S. Pat. No. 4,131,512 issued Dec. 26, 1978 entitled "Method For Detecting Microbial Pathogens Employing A Cushioning Agent". Also, in accordance with one embodiment of the subject invention, a novel method of assembling and sterilizing a lysis-centrifugation device is provided which includes:

(a) depositing a liquid cushioning agent such as disclosed in said '948 patent, and a specimen transport system in the form of solid particles within a lysis-centrifugation tube;

(b) creating a vacuum in said tube and heating said tube to the vaporization temperature of said liquid cushioning agent, e.g., about 120° C. for a sufficient time, e.g., about 30 minutes to sterilize the interior of said tube and thereafter cooling said tube to room temperature.

In addition, the system of the subject invention can be utilized in practicing the lysis-centrifugation technique as disclosed in U.S. Pat. No. 4,164,449 issued Aug. 14, 1979 and entitled "Surface Separation Technique For The Detection Of Microbial Pathogens". As an example, a specimen might be held in a container such as the lysis-centrifugation tube described above while the tube is being held for processing.

Surprisingly the novel system of the subject invention will inhibit replication of microorganisms which are contained within the specimen for a period of time up to about 72 hours after specimen collection. It is believed that replication may be inhibited for even longer periods when the subject invention is utilized, depending on the identity of the microorganisms.

The specimen transport system of the subject invention contains extremely high concentrations of specific chemical compounds which serve to neutralize antibiotics and/or normal human serum factors. These elevated concentrations cannot readily be incorporated in conventional broth systems currently used by many labora-

tories to test for microorganisms because the high concentrations of chemicals required would prove inhibitory to many potentially pathogenic organisms. However, where the specimen of interest has a high concentration of microorganisms, such as a urine specimen, the invention may be usable in conjunction with a conventional broth system, wherein the transport vessel contains the specimen and the composition of instant invention, this being diluted into the broth system when analysis is initiated. The specimen transport composition of the subject invention will effectively deactivate most antibiotics and other antimicrobial factors where a sample fluid is mixed therewith and will stabilize the viability of microorganisms of interest.

It is usually necessary that the resulting admixture of specimen and the disclosed composition be diluted on growth media at the time analysis is initiated in order that the concentrations of the deactivating chemicals be reduced to a concentration noninhibitory to microorganisms of interest. Thus, the invention is particularly useful and advantageously employed in a method in which dilution is necessary prior to microbial analysis. For example, swabs, sputums, urines, blood processed by and the lysis-centrifugation systems disclosed in U.S. Pat. Nos. 4,164,449; 4,131,512; and 4,221,948 described above generally require a high dilution factor and therefore are suitably preserved by the present invention.

As an illustration of the benefits of the instant invention, the lysis-centrifugation system as described above is an appropriate example. If the specimen transport system is included within the centrifugation tube for treating the blood sample prior to centrifugation and deposit of the concentrated microorganisms on the media, the microorganisms of interest will be protected from attack by anti-microbial factors which are present in the liquid sample such as antibiotics and serum factors which are cidal in nature. In contrast, without the instant invention, microbial pathogens may be destroyed within the centrifugation tube prior to processing resulting in undesirable false negative analysis results of cultures or inaccurate quantitation. The basic benefit of use of the subject invention can be more graphically illustrated by the following theory. Septicemia, microorganisms in the bloodstream with clinical signs of shock, disseminated intravascular coagulation (clotting) and elevated temperature (fever), hypotension, etc., does not imply that the blood-stream itself is infected. In this theoretical model, there is primary infection elsewhere such as the kidneys, a lung, or the like, and the micro-organisms are being seeded at a given rate into the bloodstream. The immune system and/or antibiotics are eliminating the microorganisms at a fixed rate. A patient survives a septic crisis if and only if the seeding rate is less than the rate of clearance.

Thus, based upon this theoretical model, conventional blood culture systems will yield a significant number of false negative cultures because once the specimen is drawn, microbial seeding from the primary source ceases to the specimen, but the antimicrobial factors present in the patient's blood are still active. Hence, during transport to a laboratory for processing, these factors may kill the viable organisms that were present at the time of draw, and therefore, render the test negative. This concept becomes especially important for immunologically competent patients and those who are on a broad spectrum of antibiotics. Thus, the practice of the improvement of the subject invention in conjunction with the lysis-centrifugation system is to literally

preserve the microbial status of the blood sample by instantly blocking the known deleterious action of the immune system and bactericidal antibiotics prior to dilution of these factors on agar plates which is an inherent feature in the lysis-centrifugation method.

The employment of the specimen transport system in urine analysis will involve the presence of the specimen transport system in the micturition receptacle from which a clinically appropriate aliquot of urine may be removed for direct microbial analysis. Thus, the practice of the subject invention is to literally preserve the microbial status of the urine sample by instantly blocking the known deleterious action of bactericidal antibiotics and by acting as a bacteriostatic agent even in the absence of antimicrobial agents.

The employment of the instant invention with throat culture swabs, vaginal swabs, tissue, bone-marrow and other specimens similarly advantageously preserves the microbial integrity of the specimen.

BRIEF DESCRIPTION OF DRAWINGS

This invention can be more easily understood from the study of the drawings in which:

FIG. 1 is cross-sectional view of a centrifugation article which can be used to practice the subject invention;

FIGS. 2-9 depict steps of a method for detecting microbial pathogens which can employ the subject invention; and

FIG. 10 depicts another embodiment of the subject invention which comprises a device for collecting and transporting body secretion samples.

FIG. 11 graphically depicts the preservation of microbial integrity of a specimen in the presence of an antibiotic in the first hour with the subject invention as compared to conventional systems (detailed in Example V).

FIGS. 12-16 graphically depicts the preservation of microbial integrity of a specimen in the presence of an antibiotic over a four hour time period as compared to conventional systems (detailed in Example V).

FIG. 17 is a cross section of one embodiment for collecting and transporting samples.

FIG. 18 is a cross section of an embodiment of FIG. 17 after a sample has been drawn.

FIG. 19 is a cross section of an embodiment of the invention incorporating a puncturable closure member, 50 useful with the embodiment of FIG. 17.

FIG. 20 is a cross section of an embodiment for collecting and transporting samples showing an alternative piston surface and shaft detachment.

FIG. 21 is a cross section of the embodiment of FIG. 17 as it might be used in drawing a fluid sample from a sample reservoir.

FIG. 22 is a cross section of another embodiment for collecting and transporting samples as it might be used as a vacuum draw sampler.

FIG. 23 is a cross section of another embodiment for collecting and transporting samples showing the specimen transport system in a powder state.

FIG. 24 is a cross section of the embodiment of FIG. 20 showing an alternative locking method.

FIG. 25 is a cross section through line A—A in FIG. 24.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Initially, it should be noted that as used herein, the unit designation "ug" signifies micrograms.

Although not the only use for the invention, by way of explanation, embodiments of the invention will be described as they would typically be used in collecting, preserving, and transporting urine or blood samples.

Referring to FIG. 17, an embodiment of the invention may be described as an apparatus similar in shape to a typical hospital syringe having a container 210, a piston 230, and a shaft 220. Closure member 250 may take the form of a screw on cap (FIGS. 17, 18, 19, and 21), or a puncturable stopper (FIG. 23). An advantage of twisting the cap on, rather than using a stopper, is that a twisted or screwed cap does not have to be popped off. This reduces the chance of contamination of the testing personnel or the sample, or creating aerosols and other potential hazards in the air. When closure member 250 does take the form of a cap, it has a sealable aperture 270 contained therein.

Composition 260 is a composition, effective for preserving the microbial integrity of the specimen, and may be designed to retard the increase or decrease in the number of organisms present in the sampled fluid. Composition 260 may be in any state such as a tablet, a powder, or a liquid; and a preferred composition, called a specimen transport system herein, is described in more detail below.

Referring to FIG. 21, when taking a urine sample, a tube 285 is placed over aperture 270 and inserted into specimen cup 282 which contains the urine to be sampled. Shaft 220 is drawn back, bringing piston 230 along and pulling urine into the cylinder. Composition 260 dissolves into the urine in the cylinder. Once a sufficient sample has been drawn, tube 285 may be removed and dropped into the specimen cup, thereby lessening the chance that urine from tube 285 will contact any people. Next, cap 290 may be placed over aperture 270 to close container 210 (FIG. 18).

In FIGS. 17, 18 and 21, a means for removing shaft 220 is shown in the form of a narrow portion 240 of shaft 220, and as shown in FIG. 18, it allows the sample taker to break shaft 220 inside container 210. The remover means allows the entire apparatus to stand on a table or be placed in a rack. In FIG. 20 and FIG. 22 the shaft remover takes the form of a threaded connection 241 between piston 230 and shaft 220. Removal of shaft 220 allows the apparatus to be used in applications normally requiring test tubes or other containers which may be stood in a rack or used in a centrifugation device.

Now referring to FIG. 19 and FIG. 22, an embodiment for blood sampling is shown. Puncturable membrane 100 may be provided for sealing aperture 270. Piston 230 is drawn back, thus creating a low pressure area in the cylinder. A lock 215 may be provided which anchors piston 230 and preserve the low pressure area. Once piston 230 is in place, shaft 220 may be removed and discarded. Lock 215 may also take the form of snapping members 252 and 254 as shown in FIGS. 24 and 25.

Again referring to FIG. 22, needle 277 is connected to a fluid source to be sampled. Examples of sources would include an intravenous connection to a patient's vein or artery, a traditional syringe which has drawn a sample, or a tube extending into a specimen cup containing fluid. Needle 277 may puncture membrane 275,

and fluid will be drawn into the cylinder by the low pressure. Needle 277 may then be removed, and membrane 275 will again seal. Cap 290 may then be placed over aperture 270 for protection of the membrane. Composition 260 dissolves into the fluid, and the specimen may be transported to the lab for tests. Once in the lab, concave surface 280 allows the specimen to be centrifuged in the apparatus.

Now referring to FIG. 23, an embodiment for taking samples through a needle 277 is shown wherein closure member 250 takes the form of a pliable stopper, the composition 260 is in a powder state, and container 210 is made from glass. The pressure in container 210 is lower than the outside pressure. Needle 277 punctures pliable stopper 250, fluid enters container 210, and mixes with composition 260. Needle 277 is then withdrawn, and pliable stopper 250 seals the puncture. The device may then be taken to a lab for testing.

Choice of material for the container is not critical; for example, glass or plastic may be used. Glass holds a better vacuum than plastic, but plastic does not break as easily. Other containers which may be used include: general syringes, special purpose syringes such as described in U.S. Pat. No. 4,459,997 to Sarstedt, and glass vacuum tubes adapted to be punctured by a needle 20 through a pliable stopper.

In embodiments using a powder or liquid state composition, a one way valve may be used to prevent the composition (or fluid which has come in contact with the composition) from flowing out of the aperture. In the case of a powder, a screen may be used to keep the powder in the cylinder, while still allowing the fluid to pass into the container.

DETAILED DESCRIPTION OF THE SPECIMEN TRANSPORT SYSTEM

In a preferred embodiment of the invention, the composition effective for preserving the microbial integrity of the specimen comprises the novel specimen transport system of the subject invention, including specific chemical agents at relatively high concentrations which will deactivate antimicrobial factors such as antibiotics and the cidal agents within a specimen such as normal human blood, among others. The specimen suspected of containing microorganisms of interest may be a fluid such as blood or urine or a semi-solid or solid from which microorganisms are collected and suspended in an aqueous solution. This may be done, for example, by wiping a sterile swab against a solid surface of interest, retaining the swab and placing the swab in a suitable solution effective to sustain viability of microorganisms of interest. As another example, muscle tissue may be transported to the laboratory for later analysis for microorganisms of interest by taking a portion of said tissue and placing it in an aqueous receiving solution which will allow permeation and diffusion into the tissue to preserve any microorganisms in said tissue. Effective nutrients to sustain viability of microorganisms of interest are to be present in the transporting media. "Effective nutrients" to be added to a specimen may be anything from sterile, distilled, deionized water to a complete commercially available broth for microorganism growth depending on the nature of the specimen and the identity of the microorganism of interest. The criteria for being "effective" is the ability to sustain the viability of the microorganism of interest from the time of specimen collection (t_0) to initiation of specimen analysis (t_1) sufficiently, in the presence of the bac-

teriostatic agents added as a part of the specimen transport system of the present invention, so at least some of the microorganisms of interest alive in the specimen at t_0 will be able to replicate at t_1 . In the majority of instances, the survival of microorganisms from t_0 to t_1 will be at least 50% and often over 80% with the use of the present invention. However, advantages are provided by the instant invention over the art even if survival rate is not high since the survival of microbial species to t_1 is improved by this invention, leading to better identification and antibiotic susceptibility testing than ever before possible.

In some cases, the effective amount of nutrients will be only pure water, for example where the specimen is not inherently aqueous. What will comprise an effective amount of nutrients to be added depends not only on the nature of the specimen but the identity of the microorganism of interest. In addition a proper balance must be achieved between supplying nutrients effective for microbial replication and preventing the replication of the microbes during specimen transport with bacterio-static agents. Different microorganisms have different nutritional needs. The nutrients supplied in connection with the instant invention should allow the microorganisms of interest to survive until t_1 , so that when the specimen is diluted upon growth media (such as an agar plate) so that the factors in the instant invention inhibitory of replication of said microorganisms of interest are no longer effective, the surviving microorganisms of interest will be able to replicate so that testing and identification may proceed.

For example, neither blood nor urine will generally require addition of nutrients to accomplish the results described above as each inherently contains sufficient nutrients which microorganisms of general interest need over transport time periods. However, when microorganisms of interest have been collected by means of a tool to which microorganisms become attached, such as for example a swab, effective nutritional components must be supplied in conjunction with the bactericidal agents. A swab is commonly used to collect specimens from patient's throats, for example. In addition, it may be desirable for certain microorganisms of interest to add nutrients even to specimens such as blood and urine to prolong viability. Specific examples below indicate the use of effective nutrients in the specimen transport system of the instant invention.

A growth base effective for supporting general nutritional needs of microorganisms of interest without inhibiting them is desirably added if the specimen itself does not inherently contain this effective nutrition. One effective growth base is Mueller-Hinton Broth (available from BBL Microbiology Systems, Cockeysville, MD 21030). This consists of Beef extract (3 g/l) Acid Hydrolysate of Casein (7.5 g/l) and starch (1.5 g/l). Another effective growth base is Tryptic Soy Broth (available from BBL Microbiology, Cockeysville, MD 21030). The composition of the growth base chosen should be noted so that if such growth base contains a portion of effective nutrients that would otherwise be added separately, the amounts will be adjusted so that the total concentration of the particular-nutrient will be known. For example, it may be desirable to add starch to the nutrient medium especially if Haemophilus is an organism of interest. Mueller-Hinton Broth contains starch, so the amount added will take the Mueller-Hinton contribution into account.

In the specimen transport system of the instant invention, a combination of effective nutrients and replication inhibitors is achieved which provides nutrients to microorganisms of interest, yet inhibits replication of all microorganisms in the specimen to preserve the microbial integrity of the specimen. In combination with appropriate replication inhibitors, it has been found that about 0 to about 10% (w/v of growth base per total volume of specimen plus transport system) is effective where it is necessary to add nutrients. A preferred range is 0.1% to 5.0%. Even more preferred is from about 1% to about 3%.

Starch is preferably employed in connection with throat cultures, where *Haemophilis* is a microorganism of interest, since starch appears to aid *Haemophilis* survival, however starch is not considered necessary for all specimens or microorganisms of interest. When starch is desirable, it has been found effective from about 0.005% to about 2.0% (w/v of growth base per total volume of specimen plus transport system). More preferred is 0.01% a range from about to about 1.5%. Most preferred is a range from about 0.1% to about 1.0%.

Agar is also a desirable, but not necessary, nutrient. It provides a surface for growth and keeps microorganisms dispersed in a fluid medium. The range of agar employable is from about 0 to about 5% (weight per volume of specimen and specimen transport system total), preferably 0.5% to about 2% and most preferably 0.1% to 1.0%.

The effective nutrients for a specimen suspected to contain *Haemophilis* includes hemoglobin. Hemoglobin also improves *Streptococcus pneumoniae* and so is desirable when this is the organism is of interest. Surprisingly, when hemoglobin is utilized for the transport system of the instant invention, no source of NADP (nicotinamide adenine dinucleotide phosphate) need be added to support *Haemophilis*. It is known that some *Haemophilis* strains require a so-called "x" factor and a so-called "v" factor (NADP). Hemoglobin supplies the "x" factor, but the need for adding an exogenous source of NADP is not evident when the instant invention admixture is employed.

Deactivation of antimicrobial factors is also part of the function of the instant invention. For example, in accordance with one embodiment of the invention, blocking agents for aminoglycoside antibiotics and polymyxin B are included within the specimen transport system. Typical aminoglycoside antibiotics include gentamicin, tobramycin and amikacin. The aminoglycosides and polymyxin B all have net positive charges. When this charge is blocked, these compounds lose their potency. Therefore, in accordance with one embodiment of this invention, a blocker for this positive charge is included within the specimen transport system. A preferred compound is sodium polyanetholsulfonate. The sodium polyanetholsulfonate will inhibit the action of aminoglycosides and polymyxin B in direct proportion to its concentration. Surprisingly, it has been found that the concentration needed to completely inhibit these antibiotics is a concentration of at least approximately 0.06% weight/volume of specimen of sodium polyanetholsulfonate, a concentration taught to be toxic by the prior art. Another such blocker compound is sodium amylosulfate. The specimen transport system of the subject invention contains sufficient sodium polyanetholsulfonate to result in between about 0.06% to about 6.0% and preferably from about 0.1% to about

5 2.0% (by weight of the SPS based upon the total weight of sample fluid and specimen transport system composition). Most preferably, SPS is added in the range of from about 0.3% to about 1.0% (by weight of SPS based upon the total weight of sample fluid and specimen transport system composition). The "toxic" effect of sodium polyanetholsulfonate to certain microorganisms has been eliminated in the instant invention by employing it in a method where subsequent dilution on 10 growth media to an approximate final concentration of 0.03% or less (by weight sodium polyanetholsulfonate on the medium).

The concentration of SPS employed in the instant invention is one sufficient to block the action of aminoglycosides, streptomycin and polymyxin B, as previously discussed. SPS at high concentration is also effective in controlling the replication of some microorganisms from t_0 to t_1 and as a result, lowering the effectiveness of antibiotics which require microbial replication for activity. It is surprising that SPS can be used in a system involving the detection and identification of microorganisms since the prior art teaches that SPS is toxic to microorganisms at concentrations exceeding 0.03%. Such low concentrations of SPS as are taught to be nontoxic in the prior art would be ineffective in the instant system to accomplish the desired results.

The specimen transport system of the subject invention preferably contains a water-soluble component effective for blocking the action of penicillin and cephalosporins, and which in combination with other components of the specimen transport system will exert a bacteriostatic effect on the replication of microorganisms in the specimen without exerting a cidal effect on the microorganisms of interest. Sulphydryl-containing compounds such as L-cysteine, N-acetyl-cysteine, thioglycolate, glutathione and mercaptoethanol are suitable antibiotic inhibitors for the penicillin and cephalosporin classes. However, it has now surprisingly been found that the concentrations used in the past are suboptimal to achieve the desired goal of antibiotic blockage, and that higher concentrations, taught to be toxic to microorganisms in the prior art, may be used in a method for preserving the microbial integrity of a specimen with the advantage of both blocking antibiotic action and acting as a bacteriostatic agent in combination with other specimen transport system components. Another effective antibiotic blocker that may be employed in the specimen transport system of the subject invention is an enzyme specific for the antibiotic. If utilized, enzyme is employed in conjunction with a sulphydryl-containing compound in the present invention as it has been found that the combination of enzyme with the other specimen transport system components exerts an effect not possible with enzyme alone.

It is preferred that the component effective for blocking the action of penicillins and cephalosporins be available in a dry form, such as a salt or a freeze-dried form so that it may be used in a dry admixture. However, liquid blocking components such as mercapto-ethanol may be utilized if desired in a liquid version of a specimen transport system, or as part of liquid specimen diluent supplied in conjunction with a dry admixture.

One or more sulphydryl-containing compounds may be used in combination, particular combinations being preferred.

L-cysteine is the preferred inhibitor of penicillins and cephalosporins present in the specimen transport system in an amount to result in from about 8.2 uM to about

8.25 mM in the combined sample fluid and specimen transport system. The most preferred amount differs according to the specimen. With urine specimens, it is preferred to employ a range from about 0.82 mM to about 41.3 mM, most preferably 4.1 mM to 24.8 mM. With throat cultures and other specimens, the preferred range is from about 0.82 mM to about 24.8 mM and most preferably from about 0.82 mM to about 8.3 mM. In a particularly preferred embodiment, the specimen transport system of the subject invention contains a synergistic mixture of thioglycolate and cysteine with cysteine contained therein in an amount from about 8.2 uM to about 82.5 mM and thioglycolate contained therein in an amount from about 0 to about 42.5 mM (molar equivalents based on the molecular weight of thioglycolic acid as the active ingredient). This combination will deactivate the penicillins, cephalosporins and some aminoglycosides very effectively and also reduce the viscosity of the thus formed system and increase shelf life of the dry admixture. Thioglycolate and similar compounds by themselves cause an undesirable increase in viscosity of the transported specimen. It has been found, however, that the above-described combination of cysteine and thioglycolate results in much lower viscosity after lysing of blood, for example. In addition, the combination allows proportions of thioglycolate that are less toxic to the fastidious microorganisms. Another advantage is that cysteine is easily oxidizable and the presence of thioglycolate helps maintain the cysteine in a reduced state in the course of preserving the microbial integrity of the specimen, for example, during the preparation of the lysis centrifugation tube and for shelf life stability of the specimen transport system admixture. An example of the combination of the cysteine and thioglycolate that can be used in a centrifugation tube as set forth in U.S. Pat. No. 4,212,948 includes an initial concentration of cysteine of 1.2% and thioglycolate of 0.1% by weight in the sample fluid and specimen transport system and once finally diluted on growth media as disclosed in said patent a final concentration of cysteine of about 0.018% and thioglycolate of about 0.002% by weight. It is noted that because of the propensity of the cysteine to oxidize, it is desirable to add the cysteine during the manufacture of a centrifugation tube during the last step prior to tube evacuation and autoclaving. The purity of the cysteine is important. Because of the high concentration of cysteine required in the specimen transport system, this compound should have a purity of greater than 95%. If one uses cysteine which is contaminated with cystine, the cystine will precipitate out during the processing of blood. Since, the cystine precipitate resembles small colonies of microorganisms on the agar plate, this is an undesirable property. The inclusion of the thioglycolate and cysteine combination has a secondary effect in that it will protect anaerobic microorganisms, e.g., clostridial species, from being poisoned by the oxygen present in the blood specimen during transport of the specimen to the laboratory. This is due to the fact that the thioglycolate and other sulfhydryl compounds are excellent oxygen scavengers. Cysteine is much more effective than other sulfhydryl compounds in blocking the cidal action of penicillins, cephalosporins and some aminoglycosides on a gram or molar basis, and as mentioned, an additional benefit of the presence of the cysteine is that it will reduce the viscosity of lysed blood which improves the sedimentation of the microorganisms in a centrifugation tube. Preferably, the free base

form of cysteine is utilized to prevent the necessity for addition of high concentrations of pH adjuster such as would be required with cysteine-HCl. However, the latter may be used.

If it is desired to utilize another sulfhydryl compound rather than cysteine, and not in conjunction with cysteine, appropriate concentrations to achieve an effect to simulate cysteine's effect as closely as possible may be utilized.

Thioglycolate may be used in a blood specimen in the range of from about 4.4 mM to about 43.8 mM, preferably from about 8.8 mM to about 35.1 mM and most preferably from about 17.5 mM to about 30.7 mM.

Glutathione is effectively used in blood from about 1.63 mM to about 16.3 mM, preferably from about 3.25 mM to about 13.0 mM and most preferably from about 6.5 mM to about 11.4 mM.

For specimens other than blood, it is preferred to use higher amounts of thioglycolate or glutathione. Thioglycolate is effectively employed from about 4.4 mM to about 52.6 mM, preferably 8.76 to 43.8, and most preferably from about 17.5 mM to about 35.0. Glutathione is preferably employed from about 1.6 mM to about 19.5 mM, more preferably from about 3.25 mM to about 16.3 mM and most preferably 6.51 mM to about 13.0 mM.

In accordance with another embodiment of the subject invention, deactivators for sulfa compounds are present in the specimen transport system. It is believed that the sulfa compounds exert their antimicrobial action by interfering with the folic acid pathway of bacteria. This pathway is essential for the synthesis of the nucleic acids which are the primary compounds of microbial DNA. Accordingly, preferably para-aminobenzoic acid (PABA) may be added to the specimen transport system as a competitive inhibitor of sulfa compounds. The preferred concentration of PABA is in the range of from 5 micrograms per milliliter to about 500 micrograms per milliliter and the most preferred range is in the range of from about 10 micrograms per milliliter to about 100 micrograms per milliliter of the combined sample and specimen transport system. However, the inhibition of replication provided by the combination of the other specimen transport system components may make the addition of PABA necessary only in circumstances where very high sulfa compound concentrations are present or where it is desired to extend the hold time of specimens to the extent that the sulfa drugs begin to exert a cidal effect on the microorganisms of interest.

The specimen transport system of the subject invention can also contain enzymes which react with and deactivate certain antibiotics, for example, beta-lactamase, and penicillinase. Usually from about 1 to about 20 units of activity of such enzymes will be effective in the system to provide the blocking effect in combination with the other components of the specimen transport system. Example XV shows the synergism achieved with employment of enzyme with other specimen transport system components.

The specimen transport system of the subject invention can include other compounds, depending upon the usage of the system, for example, the system can contain lysing agents such as purified saponin disclosed in U.S. Pat. No. 3,883,425 issued May 13, 1975 and entitled "Detoxification Of Saponins" when it is desired to process blood. The composition can also contain anticoagulant such as citrate or ethylenediamine-tetraacetic acid (EDTA).

The antibiotic blockers of the instant invention, in combination, serve as bacteriostatic agents. In addition, it may be desirable to add additional bacteriostatic agents to prevent the replication of all microorganisms in the specimen. The bacteriostatic agent chosen should be noncidal to microorganisms of interest, as previously defined. The choice of bacteriostatic agents will be dependent on the type of specimen and the identity of the microorganism of interest. Also, it may be impossible or highly unlikely that certain microorganisms could exist in particular specimens so that there would be no need to employ a particular bacteriostatic agent directed toward controlling the growth of that certain microorganism in the particular system. Thus a carbohydrate, a sugar or salt such as sodium chloride or its equivalent is desirably employed to increase the hypertonicity of the aqueous specimen or specimen receiving fluid with respect to urine specimens, swab collected specimens, and other specimens in order to control the replication of the more rapidly growing organisms, for example Enterobacteriae and Proteus. Suitable salts include sodium or potassium chloride, ammonium salts such as $(\text{NH}_4)_2\text{SO}_4$ and NH_4NO_3 and other salts of nitrates, sulfates, acetates and admixtures thereof. Suitable sugars include sorbitols, mannitol, glucose and the like. Preferably, a sodium or potassium chloride is utilized in the range of 0-171.1 mM, preferably from about 8.5 mM to about 136.9 mM, and most preferably from about 17.1 mM to about 85.5 mM in the specimen and specimen transport system admixture combined.

It may be desirable to add a substance effective for inhibiting the replication of gram positive microorganisms without being cidal to microorganisms of interest. For example, *Streptococcus faecalis* and *Streptococcus agalactiae* may mask the presence of *Streptococcus pyogenes* because the two former organisms are fast-growers. Since *S. faecalis*, *S. agalactiae* and *S. pyogenes* are all gram positive, it is not desirable to employ a substance cidal toward the gram positive class in the specimen transport system as *S. pyogenes* would be killed along with the other gram positive organisms and thus could not be isolated. It has now been found that effective growth inhibition of microorganisms without death can be achieved by the combination of the specimen transport system components plus a dye such as brilliant green or malachite green. Also effective in combination with the other system components is oxgall (dehydrated fresh bile). Brilliant Green is utilized in the range of 0 to 4.1 uM. It is preferred that it be added from about 100 nM to about 3.3 uM. Most preferred is a range of about 200 nM to about 2.1 uM. If Malachite Green is employed, the concentration in the final specimen solution should be from about 0 to 5.5 uM, preferably 100 nM-4.4 uM and most preferred 2.7 uM-27.4 uM. Other dyes may be employable at concentrations inhibitory to gram positives without being cidal, the inhibition reversible upon adequate dilutions.

Oxgall is utilized, in an amount from about 0 w/v to about 0.002% w/v, preferably 0.00005% w/v to about 0.016% w/v, and most preferably from about 0.0001% w/v to about 0.001% w/v. Since oxgall is literally dehydrated fresh bile from oxen gall bladders, no certain molecular weight or consistency between preparations is possible. Therefore, the amounts given are estimated based on preparations purchased from Difco, catalogue #0128-02.

In some specimens, it may be desirable to add additional bacteriostatic agents. It has been found that cal-

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cium propionate, methyl paraben, potassium sorbate, sodium nitrate, and sodium benzoate appropriately work in the transport system as a bacteriostat primarily for *E. coli*, *Klebsiella*, and *Enterobacteriaceae*. These agents are generally effective from about 0.1% to about 10% w/v preferably 0.01% to about 8.0% w/v, and most preferred 0.1% to about 5% w/v. Calcium propionate is most preferred. Based on the molar equivalents of propionic acid as the active ingredient, it is utilized from about 0 to about 42.1 mM, preferably from about 42 uM to about 33.7 mM, and most preferably 421.4 uM to about 21.2 mM.

It is desirable to keep the pH of the system at about 6.5-7.5. Therefore, it may be appropriate to buffer the specimen with an effective pH buffer after adjusting specimens which are markedly acidic or basic. The pH of the urine is one indication of the body's natural defense mechanism. Thus, extremes of pH (acidic) may kill microorganisms of interest in the specimen before analysis. Extremes of pH may indicate rapid replication of microorganisms which may mask the microbial integrity at time t_1 . However, the pH buffer must be compatible with the system. A preferred pH buffer is sodium bicarbonate. For urine, it may be present in the range from about 1.2 mM to about 238.0 mM, preferably from about 2.4 mM to about 59.5 mM. The concentration may be modified to achieve the desired buffering result. For other specimens, not including blood which does not generally require a buffer, the concentration may range from about 0 mM to about 60 mM, preferably from about 0.6 mM to about 24.0 mM depending on the needs of pH adjustment.

The specimen transport system chemical component is preferably a dry admixture which is employable in a specimen collection vessel for aqueous specimens, and which will dissolve in said aqueous specimen when the specimen is introduced into the collection vessel. It is most preferable if the collection vessel is utilized for specimen transport and perhaps other processing steps to reduce manipulation of the sample and risk of contamination. An example of collection/processing vessel can be seen in Example XVI. An example of use of the dry admixture in connection with urine may be seen in Example XI. It is more convenient to employ a dry, water-soluble, admixture in a collection vessel for most of the specimen transport system components. It is highly desirable to employ L-cysteine or any sulfhydryl-containing substance employed in a dry admixture to increase shelf-life of the specimen transport system admixture. Where a liquid sulfhydryl compound, such as mercaptoethanol, is employed it is desirable to provide a closed container with an inert atmosphere, such as N_2 gas, to prevent oxidation.

In specimens which are not inherently aqueous, or which are collected using an absorption device such as a swab, it is necessary to employ an aqueous fluid as part of the specimen transport system. This aqueous fluid comprises an effective diluent which in combination with the dry admixture components will preserve the microbial integrity of the specimen. All the specimen transport system components may be put in the dry admixture with the exception of agar, an optional nutrient which may be desirable for some microorganisms of interest and inherently liquid substances such as mercaptoethanol. Agar must be pre-dissolved with adequate heat in an aqueous solution. In one embodiment of a swab-collected specimen transport system, nutrients comprising growth-supporting broth and agar will be

employed so that an appropriate volume of aqueous solution for receiving the swab will contain effective amounts of the broth and agar. In this embodiment, a compartment in the device for receiving the swab will contain an aqueous receiving fluid, the compartment being breakable by the swab to release the liquid so that the dry admixture of specimen transport system components will be mixed with, and dissolved in, the aqueous broth-agar solution near the time the swab/specimen is collected and placed in the specimen transport device. It may be practical to add certain components of the specimen transport system to an aqueous receiving solution rather than a dry admixture because of the low concentrations of the components required. An aliquot of a concentrated stock solution of the component might be added to the aqueous receiving solution rather than admixing a small amount of dry component with the dry admixture.

Thus, in one embodiment of a specimen transport system for specimens collected by swab, an aqueous receiving solution is prepared according to the following method.

DILUENT PREPARATION

Preparation of stock solution

- a. Preparation of diluent without Brilliant Green:
Mueller-Hinton Broth (MHB) 4.4 g (BBL; Cockeysville Md)

Agar 0.2 g

Starch 0.8 g

100 ml H₂O

Autoclave for 15 minutes at 121° C.

Store 25 ml in 50 ml sterile plastic conical tubes in 4° C. cold room

- b. Preparation of diluent with Brilliant Green:

Mueller-Hinton Broth 4.4 g

Agar 0.2 g

Starch 0.8 g

75 ml H₂O

Boil the mixture, then add 25 ml 20 ug/ml

Brilliant Green (2 mg/100 ml H₂O). Autoclave in 100

ml aliquots for 15 minutes at 121° C. The color should be lime green as it cools to room temperature.

Store 25 ml in 50 ml sterile plastic conical tubes in 4° C. cold room.

- c. Preparation of 1:100 hemoglobin solution:

1. Put 0.1575 g hemoglobin* powder into beaker.

2. Put in 100 ml Deionized H₂O.

3. Put stir bar into beaker.

4. Stir solution slowly for at least 30 min. without heat.

5. Using a spatula work in any floating powder on the foam or glass back into the solution until completely dissolved. Keep doing this until all powder is dissolved.

6. Using two filter papers (Whatman 934 AH -glass fiber), prefiltre the solution, wash filtering unit after filtering 100-300 ml. Do not filter more than 300 ml at a time.

7. Autoclave in 100 ml aliquots for 15 minutes at 121° C.

8. Store 50 ml in 50 ml sterile plastic conical tubes in 4° C. cold room.

* GIBCO Dri-Form Hemoglobin. Catalog #M00230.

d. The stock solution is 1 part of diluent mixed with 1 part of hemoglobin solution. Final concentration of stock aqueous receiving solution:

1:200 hemoglobin - 0.07875%
MHB full-strength - 2.2%
Starch - 0.55% (0.15% is from MHB full-strength)
Agar - 0.1%
Brilliant Green - 0.00025% (2.5 ug/ml)

A dry admixture of L-cysteine, SPS, thioglycolate, sodium chloride and calcium propionate to provide the following concentration in the transport system aqueous receiving solution is then made:

L-Cysteine 0.25% (2.06 mM)
SPS 0.6%
Thioglycolate 0.01% (108.6 mM)
Sodium chloride 2.0% (34.33 mM)
Calcium Propionate 3.0% (20.52 mM).

The dry admixture is added to the appropriate volume of the aqueous receiving solution, preferably at the time of specimen collection.

Other embodiments will be evident from the above disclosure. It is envisioned that a fully dry admixture will be more appropriate for aqueous specimens such as urine and blood. A dry admixture and a separate aqueous receiving solution might be more preferable for a swab-absorbed specimen. Still another embodiment is a fully liquid system where the ingredients normally in the dry admixture are pre-dissolved in an aqueous receiving solution and stored in a non-oxidizing environment.

30 As an example of a device for collection of aqueous specimens, a urine collection/transport device incorporating a dry admixture such as disclosed above with an additional pH buffering substance is disclosed so that a patient may micturate directly into the collection/transport device, the dry admixture immediately mixing with and dissolving in the urine specimen. The device is then closed and transported to the laboratory. The volume of the specimen is standardized by the device so that the concentration of the water-soluble dry admixture once solubilized will be appropriate to preserve the microbial integrity of the specimen.

EXAMPLE I

Preservation of Microbial Integrated of a Reconstructed Specimen in the Absence of Antibiotics

A reconstruction specimen was prepared by inoculating a sterile cotton swab with 0.1 ml of a suspension of *Pseudomonas aeruginosa* (1×10^4 organisms per ml) (isolated and identified from a clinical specimen according to known procedures approved by the American Society of Microbiology). The swab was placed in either 5 mls of Mueller-Hinton Broth Mix [hereinafter MHBM] 2.26 Mueller-Hinton Broth (MHB) (BBL Microbiology Systems, Cockeysville, Md. 21030); 0.55% starch (0.15% from MHB); 0.10% Agar and 0.079% hemoglobin or the Specimen Transport System composition described in the following table. The results indicate that the specimen transport system was effective in maintaining the microbial integrity of the specimen. The survival rate was determined by inoculating three chocolate agar plates with 0.01 ml of treated (specimen transport system) or untreated (MHBM alone) specimen at various time points. The number of colonies which grew on each plate were counted and an average of the three plates taken. A survival rate of 1.00 indicates 100% survival, values greater than 1.00 indicate growth and values less than one indicate death.

It is evident that the specimen transport system used in the above example preserved the microbial integrity of the sample so that quantitation of the number of microorganisms of interest at 72 hours after specimen collection would be possible. Without use of the specimen transport system of the subject invention, uncontrolled growth of the organism occurred. For example, at 24 hours, the sample without the subject invention exhibited over a 58 fold increase from time of specimen collection to time of analysis. It is predictable that false positive results as to the microbial population present in the specimen at the time of collection would be obtained by a laboratory analyzing the specimen to which no specimen transport system was added. Even early as 4 hours past the time of collection, the results would be skewed.

<u>Survival Rate Over Time</u>						
	Time in Hours					
	0	4	6-8	24	48	72
With Transport System Composition*	1.00	1.02	0.79	1.08	1.02	0.66
Without Transport System Composition	1.00	2.91	3.80	58.13	58.13	58.13

*2% NaCl; 3% calcium propionate; .25% cysteine, 2.5×10^{-4} % Brilliant Green; 0.6% SPS; 0.01% thioglycolate; 2.2% Mueller-Hinton Broth; 0.55% starch (0.15% contributed by Mueller-Hinton Broth); 0.1% agar; 0.07875% Hemoglobin (All % in weight per total volume).

EXAMPLE II

Preservation of the Microbial Integrity of a Reconstructed Specimen in the Presence of Antibiotics

The reconstructed specimens were prepared as described in Example I. The same specimen transport system composition was tested. Antibiotics were added at a concentration of the anticipated average maximum serum level. A value of 1.00=100% survival. Without the disclosed invention, the microbial integrity of the specimen clearly began to deteriorate even 4 hours after the specimen was taken. In the table below, it can be discerned that false negative cultures would be highly probable. Quantitation without use of the disclosed composition would be highly inaccurate.

<u>Survival Rate Over Time</u>						
	Time in Hours					
	0	4	6-8	24	48	72
Amikacin (2/ μ g/ml) & Transport System	1.00	1.08	1.11	1.01	1.10	1.14
Amikacin (2/ μ g/ml) alone	1.00	0*	0	0	0	0
Piperacillin (60 μ g/ml) + Transport System	1.00	0.99	1.07	1.13	0.91	0.95
Piperacillin (60 μ g/ml) alone Ticarcillin (150 μ g/ml) + Transport System	1.00	0.76	0.21	0.01	0	0
Ticarcillin (150 μ g/ml) alone	1.00	0.97	1.06	1.12	1.02	1.01

*0 = no growth discernable

EXAMPLE III

Synergistic Effect of Combined Specimen Transport System Components On Preservation of the Microbial Integrity of a Reconstructed Specimen

Reconstructed specimens were prepared as described in Example I with the indicated microorganisms listed in each table below rather than *P. aeruginosa*.

It can be seen in the Survival Rate results that the combined components of the specimen transport system exert a synergistic effect compared with individual components. For example, in Table III-4, the specimen transport system held the survival over time at a relatively constant level. Growth occurred with the other individual treatments, in some cases the overgrowth of the microorganism dominating the plate (TNTC values). If multiple organisms were present as would be the case in an actual specimen, this overgrowth would be especially unsatisfactory. In Table III-1, it can be seen that SPS, NaCl or MHB when used alone did not allow quantitative survival at 24 hours.

The following were tested alone or in combination with other components:

<u>Mueller-Hinton Broth (MHB)</u>	
Beef Extract	0.3%
Acid Hydrolysate of Casein	1.75%
Starch	0.15%
<u>Mueller-Hinton Broth Mix (MHBM)</u>	
Mueller-Hinton Broth	2.0%
Starch	0.55% (0.15% from MHB)
Agar	0.10%
Hemoglobin	0.07875%
<u>Brilliant Green Mueller-Hinton Broth Mix</u>	
Mueller-Hinton Broth	2.2%
Starch	0.55% (0.15% from MHB)
Agar	0.10%
Hemoglobin	0.07875%
Brilliant Green	0.00025%

TRANSPORT SYSTEM

Brilliant Green Mueller-Hinton Broth Mix+0.25% Cysteine+0.6% SPS+2% NaCl+0.1% Thioglycolate

All numbers following organism identity indicate the culture number from the American Type Culture Collection Rockville, Maryland. SPS=sodium polyanethol sulfonate.

TABLE III-1

	<u>Survival Rate of <i>Haemophilus influenzae</i> 19418</u>			
	<u>Transport Time in Hours</u>			
	0	4	6-8	24
<u>Individual Components</u>				
0.5% Cysteine	1.00	1.02	0.98	2.05
0.6% SPS	1.00	1.10	1.07	0.08
2% NaCl	1.00	0.23	0.21	0.006
Mueller-Hinton Broth	1.00	0.87	0.84	0.04
Mueller-Hinton Broth Mix	1.00	1.64	3.60	7.85
Brilliant Green Mueller-Hinton Broth Mix	1.00	1.32	1.41	1.02
<u>Combined Components</u>				
Transport System	1.00	1.01	0.97	0.76

TABLE III-2

Survival Rate of <i>Streptococcus pneumoniae</i> 6301				
	Transport Time in Hours			
	0	4	6-8	24
<u>Individual Components</u>				
0.5% Cysteine	1.00	0.65	0.19	0.03
0.6% SPS	1.00	1.11	1.02	2.72
2% NaCl	1.00	0.94	1.21	0.72
Mueller-Hinton Broth	1.00	1.09	1.62	TNTC
Mueller-Hinton Broth Mix	1.00	1.36	3.33	8.10
Brilliant Green Mueller-Hinton Broth Mix	1.00	1.04	0.98	0.69
<u>Combined Components</u>				
Transport System	1.00	0.90	0.85	0.66

TNTC = Too Numerous To Count

TABLE III-3

Survival Rate of <i>Streptococcus pyogenes</i> 19615				
	Transport Time in Hours			
	0	4	6-8	24
<u>Individual Components</u>				
0.5% Cysteine	1.00	1.08	1.21	3.61
0.6% SPS	1.00	1.05	1.54	2.60
2% NaCl	1.00	1.11	1.12	1.82
Mueller-Hinton Broth	1.00	1.34	1.61	6.90
Mueller-Hinton Broth Mix	1.00	1.56	1.95	4.32
Brilliant Green Mueller-Hinton Broth Mix	1.00	1.53	1.86	1.54
<u>Combined Components</u>				
Transport System	1.00	0.94	0.91	0.68

TNTC = Too Numerous To Count

TABLE III-4

Survival Rate of <i>Staphylococcus aureus</i> 25923				
	Transport Time in Hours			
	0	4	6-8	24
<u>Individual Components</u>				
0.5% Cysteine	1.00	1.30	1.46	4.08
0.6% SPS	1.00	1.65	3.73	TNTC
2% NaCl	1.00	1.44	2.32	TNTC
Mueller-Hinton Broth	1.00	2.80	4.57	TNTC
Mueller-Hinton Broth Mix	1.00	1.98	14.11	48.43
Brilliant Green Mueller-Hinton Broth Mix	1.00	0.94	0.93	0.35
<u>Combined Components</u>				
Transport System	1.00	0.94	0.86	0.80

TNTC = Too Numerous To Count

TABLE III-5

Survival Rate of <i>Streptococcus faecalis</i> 2492-2				
	Transport Time in Hours			
	0	4	6-8	24
<u>Individual Components</u>				
0.5% Cysteine	1.00	1.40	2.00	TNTC
0.6% SPS	1.00	2.31	3.98	TNTC
2% NaCl	1.00	1.60	3.08	TNTC
Mueller-Hinton Broth	1.00	2.99	7.18	TNTC
Mueller-Hinton Broth Mix	1.00	11.14	24.83	60.56
Brilliant Green Mueller-Hinton Broth Mix	1.00	1.59	2.01	2.83
<u>Combined Components</u>				
Transport System	1.00	0.92	0.85	2.49

TNTC = Too Numerous To Count

TABLE III-6

Survival Rate of <i>Escherichia coli</i> 25922				
	Transport Time in Hours			
	0	4	6-8	24
<u>Individual Components</u>				
0.5% Cysteine	1.00	3.04	6.63	TNTC
0.6% SPS	1.00	4.02	18.20	TNTC
2% Salt	1.00	2.33	6.29	TNTC
10 Mueller-Hinton Broth	1.00	3.66	15.40	TNTC
Mueller-Hinton Broth Mix	1.00	4.40	28.44	59.87
Brilliant Green Mueller-Hinton Broth Mix	1.00	2.62	6.16	72.46
<u>Combined Components</u>				
Transport System	1.00	0.98	1.03	0.67

TNTC = Too Numerous To Count

TABLE III-7

Survival Rate of <i>Klebsiella pneumoniae</i> 632-2				
	Transport Time in Hours			
	0	4	6-8	24
<u>Individual Components</u>				
0.5% Cysteine	1.00	2.71	5.68	TNTC
0.6% SPS	1.00	4.87	TNTC	TNTC
2% NaCl	1.00	3.41	7.20	TNTC
Mueller-Hinton Broth	1.00	4.90	TNTC	TNTC
Mueller-Hinton Broth Mix	1.00	5.33	53.09	96.41
Brilliant Green Mueller-Hinton Broth Mix	1.00	6.87	31.98	59.17
<u>Combined Components</u>				
Transport System	1.00	1.08	1.03	0.70

TNTC = Too Numerous To Count

EXAMPLE IV

35 Preserves of Microbial Integrity of a Throat Swab Specimen from a Normal Donor to Which a Pathogen is Added

The effectiveness of the disclosed specimen transport system on preserving the microbial integrity of a throat swab specimen containing a known amount of a pathogenic microorganisms along with the normal flora found in the throat is shown in the following table. It was demonstrated that overgrowth of normal flora could mask a pathogenic microorganism in a specimen for analysis.

Normal throat flora were collected from 20 healthy donors (three swabs per donor). Each swab was then inoculated with between 10^4 - 10^6 of a human pathogen.

50 The microorganisms present on each swab were subsequently extracted at time zero by vigorous agitation into 5 ml of a selected transport system. The swabs were discarded, and the liquid portions were held at 24° C. for subsequent quantitative analysis at 0, 4, 6, and 24 hours in order to determine the relative survival of the pathogen versus overgrowth by normal flora present on the swab. The following organisms were tested: *E. coli*, *P. aeruginosa*, *S. agalactiae*, *H. influenzae*, *S. pyogenes*, *E. cloacae*, *K. pneumoniae*, *S. aureus*, and *S. faecalis*.

60 With the Stuarts transport system, overgrowth by the normal flora rendered the sample difficult to interpret within six (6) hours. The low survival observed at 24 hours (0.39) could either reflect death of the pathogen or masking of the organism by excessive normal flora.

65 Similar results were obtained with the Amies transport system. The amount of overgrowth varied depending on the pathogen under analysis. The more fastidious organisms (e.g., *Haemophilus influenzae*) were more

susceptible to overgrowth. Excessive growth of normal throat flora was effectively suppressed with the disclosed specimen transport system, which prevented overgrowth of the pathogen by normal flora in the absence of antibiotic over 24 hours.

Detectability of a Pathogen in the Presence of Normal Flora [Survival 1.00 = 100%]				
	Time in Hours			
	0	4	6	24
Normal Flora + <i>Streptococcus galactiae</i>				
Plus Specimen Transport System ¹	1.00	1.00	0.87	0.83
Normal Flora + <i>Streptococcus galactiae</i> With Stuart's system ²	1.00	1.09	3	3

¹Specimen Transport System utilized in this Example comprised an admixture of 1.5% NaCl, 2.0% cysteine, 0.6% SPS, and 0.01% thioglycolate.

²Stuart's system as disclosed in Stuart et al., "The Problem of Transport of Specimens for Culture of Gonococci," 45 Canadian J. of Public Health 73 (1954).

³Overgrowth of normal flora making accurate count difficult.

EXAMPLE V

Preservation of Microbial Integrity in a Reconstructed Specimen from T₀=0 to T₁=4 Hours in the Presence of Antibiotics

It is recommended in most manuals that a specimen be analyzed within 2 hours after collection. However, this assumed safe time period is not valid in all situations. The disclosed invention is shown to be a significant improvement over prior art transport systems which do not prevent significant deterioration of microbial integrity even within as little as 15-20 minutes.

The Amies transport system C. Amies et al., 58 Canadian J. Public Health, 296 (1957) (available from Curtin Matheson Scientific, Inc.) The formula (per liter of distilled water) is:

- sodium chloride	3.0 g	40
- potassium chloride	0.2 g	
- calcium chloride	0.1 g	
- magnesium chloride	0.1 g	
- mono potassium phosphate	0.2 g	
- disodium phosphate	1.15 g	
- sodium thioglycolate	1.0 g	
- agar	7.37 g	

Stuart's Transport Medium, 45 Canadian J. Public Health 73, 75 (1956) is the following: 6 g Bacto Agar in 1900 mls distilled water, 2 ml thioglycollic acid (Difco) brought to pH 7.2 with 1N NaOH. 100 ml 20% (w/v in water) Na glycerophosphate and 20 ml CaCl₂ (1% w/v in water) is then added. 20 ml 1% w/v CaCl₂ is added and the solution brought to pH 7.4 with 1N HCl. 4 ml 0.1% methylene blue is then added.

The specimen transport system of the instant invention depicted in the following charts was of the formula:

2%	Na Cl
.25%	L-cysteine (free base)
3%	Calcium propionate
2.5 × 10 ⁻⁴ %	Brilliant Green
0.6%	SPS
0.01%	Thioglycolate
2.2%	Mueller-Hinton Broth
0.55%	Starch
0.1%	Agar

-continued

0.7875% Hemoglobin

5 Antibiotics were added at the average maximum serum level as determined by published reports. These values are set out in Example VI, Table VI-2.

The organism/ml level was tested at each time point indicated on the graphs (FIG. 11-FIG. 16).

10 In FIG. 11, it can be seen that the microbial integrity of the reconstructed specimen containing *Enterobacter cloacae* using conventional transport systems deteriorates within 20-30 minutes in the presence of the antibiotic Tobramycin at 40 ug/ml. The specimen transport system in contrast held the count constant over time.

In FIG. 12, it can be seen that the specimen transport system exhibits superiority 4 hours after specimen collection, thus surpassing the two-hour recommendation for specimen analysis in the prior art.

15 In FIG. 13, an *Escherichia coli* reconstructed specimen is tested. The specimen transport system exhibits superiority in maintaining the microbial integrity of the specimen in the presence of Amikacin at 21 ug/ml.

20 FIG. 14 depicts the preservation of the microbial integrity of *Streptococcus pneumoniae* with the subject invention compared to conventional systems in the presence of Ampicillin at 21 ug/ml. A somewhat higher recovery in organism/ml is demonstrated.

25 FIG. 15 depicts the effect of Moxalactam 100 ug/ml in a reconstructed *E. coli* specimen. The specimen transport system was able to preserve microbial integrity beyond a two-hour transport time.

30 FIG. 16 depicts the effect of the specimen transport system on *Klebsiella pneumoniae* in the presence of Cephalothin. The Amies and Stuarts Systems received a slightly higher inoculum than the Specimen Transport System, however the former two systems still show dramatic drops in organisms/ml at 3 hours.

EXAMPLE VI

Comparative Average Microbial Integrity (SWABS)

Specimen transport was tested by obtaining microbial pathogens from the American Type Culture Collection (ATCC), Rockville, Md and inoculating multiple sterile cotton swabs with 1×10⁴ of a single pathogen. Each inoculated swab was placed in an aqueous preparation comprising 0.25% (2.06 mM) L-cysteine (free base), 0.6% SPS, 0.01% (108.6 mM) thioglycolate, 2.0% (34.22 mM sodium chloride), 3.0% (20.52 mM) calcium propionate, 2.2% Mueller-Hinton Broth, 0.55% starch; 0.1% agar; 0.7875% (1.2 uM) hemoglobin, and 2.5×10⁻⁴% Brilliant Green (0.5 uM) or the transport medium disclosed in 45 Canadian J. of Public Health 73 (1954) or Amies's Transport Medium (without charcoal) (58 Canadian J. of Public Health 296 (1967)).

35 Either a specific concentration of a selected antibiotic or no antibiotic was added to each individual aqueous preparation. The antibiotic concentration was chosen according to published values of the average maximum serum levels that would be found in patients. This level is indicated for each antibiotic in Table VI-2. (It should be noted that for urine specimens, not tested in this example, 10X the antibiotic average maximum serum level was employed). The number of bacteria in each specimen were determined at each of 4 time points in the three transport solution preparations by transferring 0.01 ml to each of three chocolate agar plates, incubat-

ing at 37° C. for 24 hours, counting the number of colonies, and calculating the number of microorganisms surviving per ml.

In the chart below, a value of 1.00 = 100% survival. Thus at 0 hours, all test specimens show a value of 1.00. A value greater than 1.00 indicates replication of the organism occurred in the transport period by the factor times 1.00 which yields that value. A value less than 1 indicates that the numbers of organism were reduced during transport (death occurred). Thus a value of 0.5 indicates a loss of half the original number of organisms. The values in the chart are averaged for the gram negative organisms tested (see chart below) and the gram positive organisms tested (see chart below) for the antibiotic classes given.

TABLE VI-1

LIST OF ORGANISMS USED FOR SPECIMEN TRANSPORT SYSTEM COMPARISONS		
	ATCC # ¹	CLINICAL STRAIN ²
GRAM NEGATIVE		
<i>Enterobacter cloacae</i>	3118-1	
<i>Escherichia coli</i>	25922	

TABLE VI-1-continued

LIST OF ORGANISMS USED FOR SPECIMEN TRANSPORT SYSTEM COMPARISONS		
	ATCC # ¹	CLINICAL STRAIN ²
<i>Haemophilus influenzae</i>	19418	
<i>Haemophilus influenzae</i>	9795 (Type B)	
<i>Haemophilus influenzae</i>	9133	
<i>Haemophilus influenzae</i>	8149	
<i>Klebsiella pneumoniae</i>		632-2
<i>Pseudomonas aeruginosa</i>		277
<i>Staphylococcus aureus</i>	25923	
GRAM POSITIVE		
<i>Streptococcus agalactiae</i>	624	
<i>Streptococcus faecalis</i>		2942-2
<i>Streptococcus pneumoniae</i>	6301	
<i>Streptococcus pneumoniae</i>	9163	
<i>Streptococcus pneumoniae</i>	10813	
<i>Streptococcus pneumoniae</i>	27336	
<i>Streptococcus pyogenes</i>	19615	
<i>Streptococcus pyogenes</i>	12344 (Type 1)	
<i>Streptococcus pyogenes</i>	12383 (Type 3)	
<i>Streptococcus pyogenes</i>	12385 (Type 4)	

¹American Type Culture Collection, Rockville, Md.²Clinical isolate identified according to methods approved by the American Society of Microbiology.

TABLE VI-2

ANTIBIOTICS USED FOR EXPERIMENTS

DRUG-MANUFACTURER	AVERAGE MAXIMUM SERUM LEVELS (ug/ml)
I. AMINOGLYCOSIDES	
AMIKACIN BASE - Bristol Laboratories	
GENTAMICIN SULFATE - Schering Corporation	
TOBRAMYCIN - Eli Lilly & Company	
II. CEPHALOSPORINS	
CEFAMANDOLE LITHIUM - Eli Lilly & Company	
CETRIAXONE - Hoffman-La Roche, Inc.	
CEFOTAXIME SODIUM - Hoechst-Roussel Pharmaceuticals, Inc.	
CEFOXITIN SODIUM - Merck, Sharp, & Dohme	
CEPHALOTHIN SODIUM NEUTRAL - Eli Lilly & Company	
MOXALACTAM DIAMMONIUM - Eli Lilly & Company	
III. PENICILLINS	
AMPICILLIN TRIHYDRATE - Bristol Laboratories	
CARBENICILLIN DISODIUM - Beecham Laboratories	
(20 <i>E. coli</i>)	
METHICILLIN SODIUM - Bristol Laboratories	
MEZLOCILLIN SODIUM - Miles Pharmaceuticals	
PENICILLIN G POTASSIUM BUFFERED - Eli Lilly & Company	
PIPERACILLIN SODIUM - Lederle Piperacillin, Inc.	
TICARCILLIN DISODIUM - Beecham Laboratories	
IV. OTHERS	
BACTRIM (Sufamethoxazole-Trimethoprim) - Hoffmann-La Roche, Inc.	
CHLORAMPHENICOL - Parke-Davis	
ERYTHROMYCIN GLUCEPTATE - Eli Lilly & Company	
GANTRISIN (Sulfamethoxazole) - Hoffman-La Roche, Inc.	
POLYMYXIN B SULFATE - Pfizer, Inc.	
TETRACYCLINE HCl - Lederle Laboratories Division	
VANCOMYCIN HYDROCHLORIDE - Eli Lilly & Company	

AVERAGED RECOVERY VALUES¹
FINAL DEVICE COMPARISONS: TRANSPORT TIME IN HOURS

	0 HR	HR	24 HR				
	S.T.S. ¹	STUART'S ³	AMIES ⁴	S.T.S.	STUARTS	AMIES	S.T.S.
GRAM-NEGATIVES							
I. AMINOGLYCOSIDES							
II. CEPHALOSPORINS	1.00	.93	.002	.003	.73		
III. PENICILLINS	1.00	.69	.20	.12	.45		
IV. OTHERS	1.00	.95	.36	.40	.80		
V. NO ANTIBIOTIC	1.00	.89	.38	.65	.91		
GRAM-POSITIVES							
I. AMINOGLYCOSIDES	1.00	.88	.30	.38	1.06		
II. CEPHALOSPORINS	1.00	.72	.65	.62	.58		
III. PENICILLINS	1.00	.96	.39	.37	.96		
IV. OTHERS	1.00	.93	.76	.77	.77		
V. NO ANTIBIOTIC	1.00	.92	1.15	1.12	1.23		
TOTAL ANTIBIOTICS	1.00	.87	.38	.41	.78		

TABLE VI-2-continued

TOTAL WITHOUT ANTIBIOTICS	1.00	.94	5.03	7.90	1.04
	24 HR		48 HR		
	STUARTS	AMIES	S.T.S.	STUARTS	AMIES
<u>GRAM-NEGATIVES</u>					
I. AMINOGLYCOSIDES	0	0	.44	0	0
II. CEPHALOSPORINS	.08	.04	.28	.03	.02
III. PENICILLINS	.11	.08	.57	.06	.03
IV. OTHERS	.21	.32	.78	.10	.16
V. NO ANTIBIOTIC	41.28	46.04	.55	33.65	39.73
<u>GRAM-POSITIVES</u>					
I. AMINOGLYCOSIDES	.15	.32	1.49	.02	.12
II. CEPHALOSPORINS	.28	.32	.41	.12	.13
III. PENICILLINS	.08	.09	1.45	.03	.03
IV. OTHERS	.52	.51	.62	.33	.24
V. NO ANTIBIOTIC	7.56	17.78	2.48	7.79	18.93
TOTAL ANTIBIOTICS	.18	.21	.76	.09	.09
TOTAL	24.42	31.91	1.52	20.72	29.34
WITHOUT ANTIBIOTICS					
<u>NUMBER OF SPECIFIC DEVICE RECONSTRUCTIONS</u>					
	S.T.S.	STUARTS	AMIES		
WITH ANTIBIOTICS	419	272	284		
WITHOUT ANTIBIOTICS	119	131	68		
	538	403	352 = 1293 Total Reconstructions		

¹The above data was generated using 19 pathogens - listed in Table VI-A. Data from all gram negative organisms was averaged separately from gram positive organisms.

²S.T.S. = Specimen Transport System

³Stuart, 45 Canadian J. Public Health 73 (1954)

⁴Aimes, 58 Canadian J. Public Health 296 (1967) (without charcoal)

Now referring to FIG. 1, centrifugation article 20 is depicted which is disclosed in the above-described U.S. Pat. No. 4,131,512 and its division U.S. Pat. No. 4,212,948, which patents are herein incorporated by reference into this application. The incorporated patents are directed to a method and apparatus which provides for improved rapid quantitative analysis of a blood sample for the presence of microbial pathogens. The blood sample is lysed and deposited on a high density water immiscible, hydrophobic, nontoxic, liquid cushioning agent and subjected to centrifugation. The microbial pathogens contained in the lysed blood sample will collect in a layer adjacent the interface of the cushioning agent and the blood sample residue, and, in this concentrated form, can easily be separated from the residual portion of the blood sample for culturing and quantitative counting. As shown, the article 20 comprises an elongated tubular centrifugation vessel 22 having a conventional injectable closure member 24 which sealably closes the upper end thereof, and an injectable closure member 26 which sealably closes the lower end thereof. Article 20 contains an effective amount of cushioning agent 28. The specimen transport system when utilized in elongated tubular centrifugation vessel 22 is deposited as layer 30 of particulate solid on cushioning agent 28. The specimen transport system can be contained within an aqueous solution within article 20, e.g., about one-half milliliter, but it is preferred that said system be in the form of solid particulate 30 powder 30. Solid particulate powder 30 is not soluble within the liquid cushioning agent 28 and has a higher shelf stability than the liquid solution formed of the ingredients. In addition, the use of the particulate solid specimen transport system allows a novel sterilization technique to be carried out within the interior of article 20 which will be herein described below. In the preferred embodiment of the subject invention, the specimen transport system is present whether in aqueous solution or layer 30 sufficient so that when a sample

30 fluid such as blood is deposited therein, the combination of specimen transport system and blood will contain from about 0.1 to about 6% by weight thereof of sodium polyanethol sulfonate; from about 0.5 to about 2.5% by weight of cysteine; from about 0.1 to about 1.6% by weight thereof of thioglycolate; and from about 5 micrograms per milliliter to about 500 micrograms per milliliter of para-aminobenzoic acid. In addition, since this particular embodiment is used for processing blood samples, the resulting total volume will also include from about 0.02 to about 1% by weight of purified saponin and from about 0.01 to about 0.5% by weight of EDTA. When the specimen transport system is in the form of an aqueous solution, the configuration vessel 22 will draw approximately 7.5 milliliters of blood. It is preferred that said specimen transport system be at least 3% by volume of the total liquid in centrifugation vessel 22 including the total quantity of the specimen transport system, the sample fluid and the cushioning agent and preferably from about 5% to about 30% by volume thereof. When the specimen transport system is in the form of particulate layer 30, the elongated tubular centrifugation vessel 22 will draw about 8 milliliters of blood. In the most preferred embodiment of the subject invention, layer 30 will contain 0.096 grams of cysteine; 0.008 grams of thioglycolate; 0.048 grams of sodium polyanethol sulfonate; 0.018 grams purified saponin; and 0.008 grams of EDTA. It is noted that the EDTA is not necessary to prevent blood clot formation so long as adequate amounts of sodium polyanetholsulfonate are present. For example, another satisfactory blood treating system (layer 30) contains 0.048 grams sodium polyanetholsulfonate, 0.08 grams cysteine, 0.009 grams thioglycolate and 0.019 grams purified saponin.

The combination of specimen transport system and urine will preferably contain from about 0.6 percent to about 2.0 percent by weight thereof sodium polyanetholsulfonate; from about 0.5 percent to about 2.5 percent by weight thereof, free-based cysteine; about 0.1

percent by weight thereof, thioglycolate; about 2.0 percent by weight thereof, sodium bicarbonate; and from about 2.5 percent to about 4.0 percent by weight thereof, sodium chloride. The sodium bicarbonate was added to the urine specimen transport system in order to adjust for the normal acidity of urine and thus attain a neutral pH. The added salt, in the form of, for example, sodium chloride, increases the bacteriostatic effect of the system in the absence of antibiotics in the urine. A free-based L-cysteine, such as ICN cysteine, is preferably substituted for the previously employed L-cysteine-HCl as the former does not produce a gaseous reaction when combined with the sodium bicarbonate buffer as seen previously in the L-cysteine-sodium bicarbonate mixture.

Centrifugation vessel 22 can be made of siliconized glass or hard plastic such as polycarbonate or polypropylene. Injectable closure members 24 and 26 can comprise rubber sealing stoppers. Injectable closure members 24 and 26 both carry indentations 24a and 26a, respectively, to enhance the ease of injection by common types of injection needles. Evacuated space 32 is maintained at a lower than atmospheric pressure at a predetermined value so that the centrifugation vessel can receive a known amount of liquid by injection through injectable enclosure member 24 without excessive pressure being built up within the interior thereof which would cause injectable closure members 24 and 26 to become dislodged from the openings within the centrifugation vessel 22.

Referring especially to injectable closure member 26 at the lower end of centrifugation vessel 22, it is noted that inner surface 34 of injectable closure member 26 is positioned at an angle with respect to the walls of centrifugation vessel 22.

It is noted that article 20 is especially designed to be utilized within an angle rotor centrifuge and that the angled inner surface 34 is a complement of the angle of the rotor. It should be noted, however, that the device of the subject invention can be utilized in a conventional swinging bucket-type centrifuge. In the latter instance, surface 34 should be perpendicular to the bottom of article 20 and is otherwise utilized in the same general manner as will be described herein below for the article 20 illustrated in FIG. 1. Surface 34 should be smooth and substantially free of interstitial spaces and crevices in which microbial pathogens could be entrapped. Further, the circular sealing area around surface 34 where the material of injectable closure member 26 meets the walls of the centrifugation vessel 22 should be tightly sealed so that the interface does not provide a large circular crevice in which microbial pathogens could become lodged.

The angle of incline of smooth surface 34 with respect to the walls of centrifugation vessel 22 is determined according to the centrifugation apparatus in which article 20 is to be centrifuged.

As discussed above, when a swinging bucket-type centrifuge is utilized, surface 34 will be positioned perpendicular to the bottom of the article 20. However, when an angle rotor centrifuge is utilized, surface 34 will carry the complement of the angle of the rotor. Therefore, in general, when the rotor angle ranges from about 60° to 10°, the angle of surface 34, or angle of incline 36 within the centrifugation vessel will range correspondingly from 30° to 80°. Thus, the angle of incline, depicted by arc 36, will generally be the complement of the angle at which device 20 rests within the

centrifuge during centrifugation. For example, the angle of incline 36 depicted in FIG. 1 is approximately 34°. Thus, for example, when article 20 is placed in an angle rotor centrifuge in which centrifugation occurs at approximately 56°, fluids contained within article 20 will be forced against surface 34 at a substantial perpendicular angle.

The amount of cushioning agent 28 employed should be sufficient to completely cover surface 34 upon centrifugation. The amount of cushioning agent utilized can vary with the parameter of the particular system chosen, for example, the stopper design, volume of residual blood and volatility of the cushioning agent utilized. A preferred amount of cushioning agent can comprise from about 3.3% to about 40% by volume based on the volume of the cushioning agent-residual blood sample mixture which is removed from article 20 and tested for the presence of microbial pathogens.

Generally, the cushioning agent of the subject invention can comprise a high density, hydrophobic, water immiscible liquid. As noted previously, the term "high density" as used herein refers to a liquid which will not be supported by the mixture of blood and blood treating fluid or any other sample fluid suspected of containing microbial pathogens in the presence of centrifugal force. In addition, the cushioning agent should be non-toxic to microbial pathogens and relatively inert with respect to butyl rubber, silicone rubber and other types of elastomers employed in the manufacture of the injectable closure members described above. The density of the cushioning agent can be in the range of from about 1.2 grams per cubic centimeter to about 2.0 grams per cubic centimeter. Generally, fluorinated hydrocarbons having the above described characteristics and having molecular weights in the range of from about 300 to about 850 are preferred. Furthermore, fluorinated hydrocarbons having the above qualities which have a vapor pressure at 77° F. and 1 atmosphere from 0.06 psi (0.3 mm Hg) to about 0.58 psi (30 mm Hg) and preferably a vapor pressure approximately equal to that of water. Therefore, cushioning agents having the above described qualities and boiling points of about 200° F. to about 420° F. (93° C.-216° C.) and preferably of about 225° F. to about 280° F. (106° C. to 138° C.) can be utilized. The cushioning agents preferably have specific heat at least equal to or greater than 0.2 g-cal/g°C at 77° F. and 1 atmosphere, and most preferably specific heat at least equal to or greater than water. The cushioning agent should also have a vapor pressure which will not disrupt the injectable closure means from the tube during manufacturing steps such as autoclaving, for example. Fluorinated hydrocarbons sold under the trade name FLUORINERT by 3M Company of Minneapolis, Minnesota, have been found to perform well as cushioning agents. Specifically, types FC-75, FC-48, and FC-43 of the FLUORINERT series have been found to be especially useful.

Although the exact function which such cushioning agents perform is not fully known, it is believed that they improve collection of microbial pathogens which have passed from suspension in a centrifuged blood sample in at least two ways. First, the cushioning agent serves to seal interstitial spaces, cracks and crevices both on the smooth surface 34 of the centrifugation vessel 22 and the interface between the walls of the centrifugation vessel 22 and injectable closure member 26. Thus, microbial pathogens which might otherwise become entrapped in such interstitial spaces, and there-

fore not recovered, are recovered with the cushioning agent 28 when it is removed from article 20. Secondly, it is believed that the cushioning agent does act to cushion the impact of microbial pathogens which are forced out of suspension in a blood sample during centrifugation. This cushioning effect reduces the danger of injury to microbial pathogens which might otherwise occur upon impact. Further, while some of the microbial pathogens may actually pass into the cushioning agent, substantially none will pass completely through it and a majority will form on its surface at the interface between the cushioning agent 28 and the blood sample and collect in a layer.

After the cushioning agent 28 has been deposited within centrifugation article 20, the specimen transport system 30 for the blood may also be deposited there.

Once the specimen transport system 30 has been deposited in centrifugation article 20, injectable closure member 24 can be put in place and space 32 evacuated to the desired lower than atmospheric pressure, e.g., 25 to 30 inches of mercury. In accordance with one embodiment, the interior of centrifugation vessel 20 is next sterilized by a novel technique. It has been found that if a centrifugation vessel is heated to the vaporization point of the FLUORINERT material therewithin, e.g., at least about 120°C and held for a sufficient time, e.g., at least about 30 minutes, the interior of the tube and the solid particulate specimen transport system 30 will become sterilized by the hot FLUORINERT vapors. Once this is done, the centrifugation vessel 20 is merely cooled to room temperature and packaged for sale, for example.

Now referring to FIGS. 2-9, an analysis sequence is schematically depicted illustrating a preferred embodiment of the subject invention. As an example, a procedure which is carried out in accordance with one embodiment of this invention for detection of microbial pathogens within a blood sample can be carried out conveniently with the following apparatus:

The above described centrifugation article 20 containing the cushioning agent 28 and specimen transport system 30. The vessel can be of 12-14 milliliters in volume.

A sterile glass syringe and one 1½ inch 21 gauge disposable hypodermic needle;

One sterile glass syringe and one 1 inch 18 gauge disposable hypodermic needle;

One ½ inch 25 gauge hypodermic needle with cotton inserted at its hub (used as a vent);

Two blood agar plates;

Two chocolate agar plates.

It is noted that with the exception of centrifugation article 20 or some equivalent article, various types of well-known laboratory apparatus and culture media can be used to carry out the novel process of the subject invention. It is particularly noted that the culture media set forth above are exemplary only and are generally preferred to be utilized for detecting the most commonly known microbial pathogens. The blood agar plates suggested are conventionally utilized blood agar plates which are basically sheep's blood and a base nutritional agent such as brain heart infusion, which is held together with an agar solidifying agent on a petri plate. The chocolate agar plate is designed to grow certain factitious pathogens, e.g., Hemophilus.

Thus, while various apparatus can be utilized in the method of the subject invention, the above list of appa-

ratus and materials can be conveniently utilized in the scope of this invention in a manner set forth below.

To utilize centrifugation article 20 set forth in FIG. 1 in the drawing, it is initially positioned so that injectable closure member 26 with its smooth angled surface 34 is at the lower end of article 20 so that the cushioning agent 28 specimen transport system solids 30 rest upon smooth angled surface 34. In practice, a mixture of cushioning agent 28 and the solid articles of specimen transport system 30 may occur due to handling so that two distinct layers may not always be present. This unstable mixture of cushioning agent 28 and specimen transport system 30 in no way adversely affects the method set forth herein since the solids forming system 30 will rapidly dissolve in the aqueous sample (blood) and separation of the two resulting liquid phases rapidly occurs upon centrifugation.

Next, a predetermined amount of a blood sample 38 drawn from the patient, for example, 8 milliliters of blood, is injected into the evacuated space or centrifugation article 20 as depicted in FIG. 3 using a common type of syringe 40. Alternately, the sample can be drawn directly into article 20 using a standard and double needle fixture supplied with conventional vacuum blood drawing devices such as sold under the mark "Vacutainer" by Becton Dickinson. Then, article 20 containing the blood sample 38, the specimen transport system 30, and the cushioning agent 28 is subjected to mixing to insure that the anticoagulants, red cell lysing agent, and the specimen transport system 30 are completely admixed with the blood sample 38. This mixing step is depicted schematically by FIG. 4. The mixing step will insure that the specimen transport system 30 containing the lysing agent will be completely admixed with and solubilized by the blood sample. This solubilizing action will assure contact between antimicrobial factors and the chemical components of the specimen transport system 30 and thus assure that any pathogens contained within the blood sample 38 will be protected from antimicrobial activity.

After the blood sample 38 has been treated in this manner, centrifugation article 20 is centrifuged to cause the microbial pathogens within the treated blood sample 42 to pass out of suspension and collect adjacent the interface of the high density cushioning agent 28 and the residual of the sample fluid. Some microbial pathogens will actually be deposited upon the sidewall of centrifugation vessel 22 adjacent the high end of smooth surface 34 at point 22a. This centrifugation step is represented schematically by FIG. 5. The speed and time of centrifugation can vary widely depending upon the construction material of centrifugation article 20 and the type of centrifugation apparatus. The centrifugation can be conveniently accomplished by imparting from between about 1500 to 6000 gravities and preferably from about 1500 to 3000 gravities to the centrifugation article 20 containing the treated blood sample 42 and cushioning agent 28. As depicted in FIG. 5, an angle rotor centrifuge is employed which places the centrifugation article 20 at an angle of 56° for example, (depicted by arc 43) during centrifugation. Thus, if smooth angled surface 34 is at a 34° angle with respect to the interior walls of centrifugation article 20, the treated blood sample 42 and cushioning agent 28 will be forced against smooth angled surface 34 at a relatively perpendicular angle during centrifugation. It is noted that when a swinging bucket type of centrifuge is employed, centrifugation article 20 will be centrifuged at substan-

tially 0° with respect to a horizontal surface. Thus, in such a case,

the angle of surface 34 will be approximately 90° and an injectable rubber closure member having a flat inner surface can be substituted for injectable closure member 26.

Once the centrifugation step has been completed, centrifugation article 20 can be removed from the centrifuge and the major portion of the treated blood sample 42 from which microbial pathogens have been separated can be removed. It is noted that, as used herein, the term "residual treated blood" or "residual blood" refers to a blood sample which has been centrifuged such that the microbial pathogens present therein have collected at the bottom of the sample, hence, leaving the "residual" portion of the sample substantially free of microbial pathogens. This step is depicted in FIG. 6. To aid in ease of removal, a vent needle 44 in the form of a common hypodermic needle with cotton in its hub, for example, is injected through injectable closure member 24. A second hypodermic needle with syringe 45 attached can then be injected through injectable closure member 26 to remove a major portion of the residual treated blood sample 42 from which microbial pathogens have been separated. For example, when the centrifugation vessel has a volume of 5 from 12 to about 14 milliliters, a 1½ inch 18 gauge 1.7 milliliters of the treated blood sample 42. As shown, it is preferred that the major portion of the residual blood sample to be withdrawn from the interior of centrifugation vessel 22 is withdrawn at a point opposite the sidewall adjacent the upper bevel end of smooth surface 34 to avoid disturbing the layer of microbial pathogens which has formed on and within the interface of the two liquids and on the sidewall of centrifugation vessel 22 adjacent the upper end of said beveled smooth surface 34. The majority of the residual blood is removed in this step; however, a small portion of the residual blood should be left in the centrifugation vessel 22 such that of the total fluid remaining, the cushioning agent comprises from about 3.3% to about 40.0% by volume. It is preferred that no more than about 20% by volume shall be said cushioning agent because greater quantities of said cushioning agent may deleteriously effect the morphology of microbial pathogen colonies in subsequent pathogen growth steps used in the process.

Once the major portion of the treated residual blood sample has been removed, both needles may be withdrawn from injectable closure members 24 and 26, and centrifugation article 20 is then subjected to a second mixing step depicted schematically by FIG. 7. However, if desired, vent needle 44 can be left in its position through injectable closure member 24 to assist in removal of the pathogen containing fluid in a later step. The second mixing step serves to resuspend microbial pathogens which have separated from the major portion of residual treated blood sample 42 and which have formed the layer described above. Resuspension of the microbial pathogens so collected in the remaining minor portion of the residual treated blood sample 42 insures greater and more uniform recovery.

Once the mixing step has resuspended, the microbial pathogens in a minor portion of the residual treated blood sample 42, the mixture of microbial pathogens in the residual treated blood sample and the high density cushioning agent can be removed from centrifugation article 20. This step is depicted in FIG. 8. As noted above, if desired, the venting hypodermic needle 44

may be inserted through injectable closure member 24 to allow easier removal of the remaining constituents. The syringe 46 with attached hypodermic needle can then be injected through injectable closure member 26 to draw out the mixture 48 of cushioning agent 28, minor remaining portion of residual blood sample 42 and microbial pathogens present therein. It is noted that particularly good recovery can be obtained if the hypodermic needle used to remove these constituents is injected at the lower end of the angled smooth surface 34. It is believed that the angle of surface 34 acts, in part, as a funnel into which the remaining fluid containing the microbial pathogens flow. This mixture 48 of high density liquid cushioning agent 28, and the remaining minor portion of the residual treated blood sample 42 with the recovered microbial pathogens should be approximately 1½ milliliters of fluid. This fluid is then distributed on appropriate growth media. This step is then schematically illustrated in FIG. 9 in the drawing. With the apparatus set forth above, the material can be distributed as follows:

Two blood agar plates can receive 0.4 milliliters of the aqueous solution and can be incubated at 36° C. in an anaerobic environment. Two chocolate agar plates can receive 0.4 milliliters of the aqueous solution and can be incubated at 36° C. in a candle jar. The growth media should be checked daily for the presence of colonies. Microbiological analysis techniques can be employed. The number of microbial pathogens in one milliliter of the blood can be determined by multiplying the number of colonies by a correction factor. This correction factor takes into consideration the recovery rate for a given organism, the volumes of blood and high density cushioning agent employed and the amount of final mixture plated. In the general example set forth above, the correction factor is 0.5.

The above procedure will result in a dilution of the remaining minor portion of the residual treated blood sample 42 to at least about 1:60 on the growth media. This will assure that any residual quantity of the chemicals within the specimen transport system will be diluted sufficiently so as to not inhibit the growth of microbial pathogens therewithin. The specimen transport system of the subject invention will either neutralize or inhibit cidal drugs. For example, the sodium polyane-tholsulfonate will generally neutralize and the cysteine will generally inhibit. Furthermore, the effect of oxygen on cysteine after removal of the sample from centrifugation vessel 22 will destroy its inhibiting effect on microorganisms. The above described dilution procedure may be necessary to dilute drugs and/or component of the specimen transport system to levels which are neither cidal nor inhibitory to the growth of microorganisms. In addition, for those antibiotics which may be present in the blood sample which exert only an inhibitory and not a cidal effect on microorganisms, the 1:60 dilution will generally prove adequate to reverse their inhibitory effect on microorganisms. An example of this class of compound is gantrisin. Thus, in genera, the 1:60 dilution will prevent the inhibiting of growth for most micro-organisms/antibiotic combinations. Nevertheless, there are certain microorganisms which are uniquely sensitive to the killing or inhibitory action of certain classes of antibiotics. For example, if one is attempting the isolation of a very sensitive strain of *S. aureus* (minimum inhibitory concentration of 0.2 micrograms per milliliter) and the blood sample contained 20 micrograms per milliliter of antibiotic not blocked by

sodium polyanetholsulfonate, para-aminobenzoic acid, or cysteine-thioglycolate, the organism would not grow on a conventional agar plate (20 milliliters of media) in accordance with the above-described method which normally deposits 0.4 micrograms per milliliter of blood sample. This combination yields a final dilution of approximately 1:60. Thus, this example would yield a final concentration of 0.33 micrograms per milliliter of antibiotic throughout the plate which would indeed inhibit the subsequent growth of the *S. aureus* strain. Furthermore, the dilution of the antibiotic is not instantaneous and initially the high levels of the antibiotic on the surface of the agar plate might exert a lethal effect. To circumvent this problem and yet preserve the known advantages of the lysis-centrifugation technique improved with the novel specimen transport system of the subject invention, one further modification is required: namely, a big petri plate. Clinical laboratories concurrently use a 150 mm × 20 mm petri plate for testing antibiotics. This plate contains between 60 ml and 80 ml of media and has 2.25 times the surface area of a conventional 100 mm × 20 mm petri plate. When one streaks the 0.4 ml blood sample uniformly on the surface of this large plate, one achieves a 2.25 fold increase in the diffusion rate and a final dilution between about 1:200 to 25 about 1:270. In the example used above, this will result in a final antibiotic concentration of between 0.1 micrograms per milliliter and 0.075 micrograms per milliliter. When this plate is used, the final concentration of the antibiotic is well below the minimum inhibitory concentration and the organism should grow in normal fashion. Thus, while the large plate need not be used in each instance, it should be used when certain fastidious organisms-antibiotic combinations are suspected, such as *S. aureus*-cephalothin.

Now referring to FIG. 10, another embodiment of the subject invention is depicted which comprises a device for collecting and transporting body secretion samples. Device 100 comprises an elongated flexible tube 102 enclosed at one end 104 and open at its opposite end 106. Cap 108 encloses the open end. Contained within the tube near closed end 104 is a crushable ampule 110 containing a suitable liquid growth media for microbial pathogens. Disposed adjacent ampule 110 is sorbent material 112 which can be any suitable sorbent such as cotton. Sorbent material 112 contains dispersed therein specimen transport system solids 114. Disposed within the open end of tube 106 is swab member 116 which comprises a handle 118 and an absorbent tip 120 for recovering body secretions from a lesion, for example.

Specimen transport system solids 114 can be the same material disclosed for use in the lysis-centrifugation vessel described above and can be present in the same relative quantities based upon the amount of solids 114 and growth media 110 and body secretion collected on absorbent tip 120 as the components described above in relation to a blood sample. In operation, cap 106 is removed and swab 116 is removed from the interior of tube 102. The swab contacts body secretion from an open lesion, for example, and is inserted again within the interior of tube 102 and cap 108 is placed over the open end 106 thereof. Thereafter, the portion of tube 102 adjacent closed end 104 is squeezed and ampule 110 is ruptured to cause the liquid growth to be released therefrom and saturate sorbent material 112 and solubilize specimen transport system solids 114. The resulting liquid containing the dissolved specimen transport sys-

tem is absorbed by the tip of swab 120 and provides a media for sustaining microbial pathogens present on the tip and also an specimen transport system for deactivating antimicrobial factors which might be present in the body secretions sorbed on the tip 120 of swab 116. The swab 116 is later removed from container 102 and microbiologically analyzed in a manner described above.

The following additional examples are given to better facilitate the understanding of this invention and are not intended to limit the scope thereof. In Examples VII-XVI:

CFU=Colony-forming units of a microorganism/ml of blood initially inoculated into the tube. Seven and one-half ml of blood are processed per tube.

% Recovery=Percentage of organism recovered in the gradient of all organisms found after processing.

S-Factor=Survival index=Number of CFU recovered from all contents in tube/number of CFU introduced: S=1 means no kill; S=0.1 means 10% survival

EXAMPLE VII

Action of Sodium Polyanetholsulfonate (SPS) on Gentamycin

Tests were made comprising the original centrifugation article disclosed and claimed in U.S. Pat. No. 4,212,948. In the original version, each tube contains 0.3 ml of FLUORINERT FC48 as cushioning agent and as a blood treating fluid 0.5 milliliters of distilled water containing 0.005 milliliters PPG, 0.008 grams of EDTA and 0.0048 grams of SPS together with 0.018 grams of purified saponin as a lysing agent. The tube was sterile with the aqueous solution having a pH of 7.4 and sufficient vacuum to draw approximately 7.5 milliliters of human blood. A second type tube was prepared except sodium polyanetholsulfonate was added to the aqueous solution in an amount to equal 0.6% by weight of the final concentration of treating fluid and blood sample. Next a series of the above described original tubes and the original tubes plus the sodium polyanetholsulfonate were tested by adding known quantities of *Staphylococcus aureus* in a blood sample containing 6 micrograms per milliliter of gentamicin. Blood was lysed, the tubes were held at room temperature (approximately 72° F.) for 2 hours prior to centrifugation to simulate clinical conditions. Thereafter the tubes were centrifuged as described above and the concentrated material plated on growth media and tested for recovery. The results are set forth below.

TABLE VII-1

SYSTEM	<i>Staphylococcus aureus</i> (ATCC 25923) Gentamicin (6 ug/ml)		
	CFU	% RECOVERY	S FACTOR
Original	133	100	.06
Original + 0.6 SPS	203	80	.9

The original tube gave an overall recovery of 6% while the SPS system gave a recovery of 72% (11.0 fold improvement).

EXAMPLE VIII

Deactivation of Ampicillin by Thioglycolate

This example was carried out in the same fashion as Example VI except the stated quantities of thioglycolate were added to the second and third series of tubes.

TABLE VIII-1

<i>Staphylococcus aureus</i> (ATCC 25923) Ampicillin (21 ug/ml)			S
SYSTEM	CFU	% RECOVERY	FACTOR
Original	196	33	.002
Original + 1% thioglycolate*	490	89	.040
Original + 6% thioglycolate	466	89	.13

*Amount of thioglycolate based upon treating fluid and blood sample.

The original tube gave an overall recovery of 0.07% recovery versus 12.5% for the 6% thioglycolate system—a 179 fold improvement.

EXAMPLE IX

Deactivation of Ampicillin and Gentamicin By a Novel Cysteine-Thioglycolate Combination

The series of runs set forth below were carried out in the same fashion as Example VII above except with the quantity and amount of antibiotic and the stated quantities of thioglycolate-cysteine which were added to the liquid blood treating material.

TABLE IX-1

<i>Staphylococcus aureus</i> (ATCC 25923) Ampicillin (21 ug/ml)			S
SYSTEM	CFU	% RECOVERY	FACTOR
Original	196	33	.002
Original + 0.5% thioglycolate* + 0.2% cysteine*	245	98	0.80
Original + 0.1% thioglycolate* + 1.2% cysteine*	696	99	1.1

*Amounts based upon total quantity of treating fluids and blood sample.

TABLE IX-2

<i>Staphylococcus aureus</i> (ATCC 25923) Gentamicin (6 ug/ml)			S
SYSTEM	CFU	% RECOVERY	FACTOR
Original	133	100	.1
Original + .5% thioglycolate*	203	100	.2
Original + .5% thioglycolate* + .2% cysteine*	287	86	.8

*Amounts based upon total quantity of treating fluid and blood sample.

The comparative data in Tables IX-1 and IX-2 above clearly demonstrate the use of the thioglycolate-cysteine combination.

EXAMPLE X

Synergistic Action of Thioglycolate-Cysteine Mixture in Lowering Viscosity of Lysed Human Blood

In each instance, 7.5 milliliters of human blood was treated with an aqueous solution containing 2.5% by weight purified saponin and quantities, if any, of thioglycolate and cysteine as illustrated in the table (based upon the total quantity of treating fluid and blood). The viscosity of each sample was measured at the temperature between 23.5° and 24.8° C. The results are set forth below:

TABLE X-1

TREATMENT (Saponin-2.5%)	VISCOSITY (Centistokes)
1. Saponin x1	4.04
2. Saponin x1 + 0.1% thioglycolate	7.28
3. Saponin x1 + 0.5% thioglycolate	7.77
4. Saponin x1 + 1.0% thioglycolate	8.56

TABLE X-1-continued

TREATMENT (Saponin-2.5%)	VISCOSITY (Centistokes)
5. Saponin x1 + 2.0% thioglycolate	8.51
6. Saponin x1 + 3.0% thioglycolate	8.46
7. Saponin x1 + 0.1% cysteine	4.56
8. Saponin x1 + 0.5% cysteine	3.46
9. Saponin x1 + 1.0% cysteine	2.89
10. Saponin x1 + 1% thioglycolate + 0.1% cysteine	5.14
11. Saponin x1 + 1% thioglycolate + 0.5% cysteine	4.30
12. Saponin x1 + 1% thioglycolate + 1.0% cysteine	3.75
13. Saponin x1 + 1% thioglycolate + 2.0% cysteine	3.43
14. Saponin x1 + 3% thioglycolate + 0.1% cysteine	6.28
15. Saponin x1 + 3% thioglycolate + 0.5% cysteine	4.58
16. Saponin x1 + 3% thioglycolate + 1.0% cysteine	3.44
17. Saponin x1 + 3% thioglycolate + 1.5% cysteine	3.82

Temperature of samples were between 23.5° C.-24.8° C.

EXAMPLE XI

Effect of Specimen Transport System in Improving Blood Specimen Microbial Integrity

The data in the following tables illustrate the following important aspects of the invention, namely:

1. In the presence of average serum levels of different antibiotics the original system can lose up to 99.7% of the original inoculum *Staphylococcus aureus* with ampicillin. For *S. aureus* 13 of 19 antibiotics killed 50% or more of organism within two hours. For *Escherichia coli* this excessive cidal action occurred with nine of the antibiotics. (See Tables XI-1 and XI-2).

2. With the new system, the highest kill rate was 70% and a reduction of the inoculum to 50% or less occurred with two antibiotics for *S. aureus* and two with *E. coli*. By adding large plates to the new device, the cidal effect observed in these four cases can be virtually eliminated ($S=0.8$ versus 0.3 ; $S=0.9$ versus 0.5 ; $S=0.8$ versus 0.5 and $S=0.5$ versus 0.3 , where $5=1.00=100\%$ survival).

In summary, the new system in conjunction with effective dilution (i.e. the use of large petri plates) is capable of effectively blocking the cidal action of blood and therapeutic antibiotics upon the bacteria present in a blood sample during transport and processing.

The data presented in Tables XI-3 through XI-10 below confirm the general effectiveness of this invention on other pathogens commonly isolated from the blood of patients suffering from septicemia.

The procedure set forth below was followed for each pathogen, using various antibiotics. Concentrated residue from each tube was plated on both small and large plates to generate the data illustrated.

A series of original lysis-centrifugation devices were assembled as described in Example VII. A second series of lysis-centrifugation devices were assembled as in Example VII with the exception that the aqueous phase was modified as follows:

0.5 milliliters of distilled water containing 0.005 milliliters of polypropylene glycol was placed into the tubes.

A total of 0.17 grams of powdered mixture was then added to each tube. The mixture contained the following components:

1.8 grams of purified saponin, 4.8 grams of sodium polyanetholsulfonate, 0.8 grams of thioglycolate, and 9.6 grams of cysteine.

The tubes were sterilized by autoclaving and had a

centrifugation as described in this specification. The concentrated residue in each tube was then plated in equal aliquots on five agar plates containing appropriate growth media which had dimensions of 100 milliliters \times 20 milliliters and growth was observed. One milliliter of the supernatant remaining after centrifugation was also plated on the five plates.

TABLE XI-1

ANTIBIOTICS***	ORIGINAL SMALL PLATES			IMPROVED SMALL PLATES		
	CFU	% RECOVERY	S FACTOR	CFU	% RECOVERY	S FACTOR
I. <i>Staphylococcus aureus</i> (ATCC #25923)						
No Drug	75	91	.9 \pm .2	745	99	1.1 \pm .2
Gentamicin (6)	133	100	.1 \pm .04	852	99	1.0 \pm .1
Tobramycin (4)	155	99	.4 \pm .2	259	95	.8 \pm .2
Amikacin (21)	76	99	1.0 \pm .3	152	99	1.1 \pm .2
Penicillin (20)	546	68	.01 \pm .01	305	96	.9 \pm .2
Ampicillin (21)	403	84	.003 \pm .003	319	94	1.0 \pm .1
Cephalothin (20)	214	100	.1 \pm .03	1177	76	.3 \pm .3
Cefoxitin (25)	158	98	.5 \pm .1	991	99	.6 \pm .2
Chloramphenicol (18)	476	99	.6 \pm .2	495	99.8	1.1 \pm .3
Tetracycline (9)	218	100	.5 \pm .4	584	100	1.2 \pm .1
Erythromycin (8)	512	100	.1 \pm .05	623	72	.8 \pm .2
Gantrisin (100)	642	93	1.0 \pm .2	441	99.7	1.0 \pm .1
Clindamycin (5)	180	99.7	1.2 \pm .9	123	78	1.3 \pm .4
Methicillin (9)	588	99.8	.9 \pm .1	408	92	1.1 \pm .3
Vancomycin (8)	286	98	.6 \pm .1	1190	52	.8 \pm .2
Piperacillin (60)	350	82	.005 \pm .007	396	99	1.2 \pm .1
Moxalactam (100)	676	100	.2 \pm .03	2345	49	1.2 \pm .4
Carbenicillin (71)	483	100	.03 \pm .02	438	99.8	1.1 \pm .3
Cefotaxime (20)	472	99	.3 \pm .1	595	98	.5 \pm .2
Ticarcillin (150)	606	50	.01 \pm .01	553	98	.9 \pm .1
II. <i>Escherichia coli</i> (ATCC #25922)						
No Drug	214	98	1.2 \pm .3	886	95	1.1 \pm .2
Gentamicin (6)	468	100	.02 \pm .01	167	97	1.1 \pm 1.0
Tontamycin (4)	129	100	.6 \pm .6	399	99	.9 \pm .2
Amikacin (21)	255	100	.05 \pm .03	420	98	.8 \pm .1
Penicillin (20)	526	99	1.4 \pm .3	206	95	1.3 \pm .3
Ampicillin (21)	490	100	.2 \pm .1	144	98	.9 \pm .2
Cephalothin (20)	413	100	.04 \pm .04	353	99	.5 \pm .1
Cefoxitin (25)	466	99	.3 \pm .06	148	89	.8 \pm .3
Chloramphenicol (18)	323	99	.8 \pm .1	395	99.6	.7 \pm .3
Tetracycline (9)	368	99	.5 \pm .04	320	98	1.1 \pm .2
Erythromycin (8)	305	99	1.0 \pm .4	212	97	1.1 \pm .3
Gantrisin (100)	140	99	1.5 \pm 1.0	282	97	.8 \pm .3
Ticarcillin (150)	574	99.8	.5 \pm .4	651	99	1.3 \pm .7
Carbenicillin (20)	1694	99.6	.2 \pm .1	417	98	1.0 \pm .2
Piperacillin (60)	364	100	.7 \pm .4	1084	96	.9 \pm .2
Cefamandole (20)	167	99.5	.4 \pm .2	153	100	.7 \pm .4
Kanamycin (14)	203	100	.1 \pm .03	176	95	.3 \pm .1
Methicillin (9)	198	99.8	1.1 \pm .2	146	99	1.1 \pm .3

All tubes were held at room temperature (20 C.) for two hours prior to processing.

The small plates contained 20 ml of agar media.

***Numbers in parenthesis represent final concentration of antibiotic used in microorganisms/ml of blood.

final pH of between 6.6 and 6.8.

Sufficient vacuum was placed in the tube to draw 7.5 milliliters of blood.

In each instance, the stated amount of specific microorganism as illustrated in tables below and antibiotic was added to 7.5 milliliters of blood. The blood was then deposited into the lysis-centrifugation tube and the tube was held at room temperature for 2 hours to simulate clinical conditions, and thereafter was subjected to

The above experiments were repeated except instead of the 100 milliliter by 20 milliliter petri plates containing media, the concentrated residue from each tube was plated on a 150 milliliter by 20 milliliter petri plate which contains between 60 milliliters and 80 milliliters of media and had approximately 2.25 times the surface area of 100 milliliter by 20 milliliter petri plate described which were used to generate the data in Table XI-1 above. The results are set forth in Table XI-2 below.

TABLE XI-2

ANTIBIOTICS***	ORIGINAL SMALL PLATES			IMPROVED SMALL PLATES		
	CFU	% RECOVERY	S FACTOR	CFU	% RECOVERY	S FACTOR
I. <i>Staphylococcus aureus</i> (ATCC #25923)						
No Drug	+	+	+	+	+	+

TABLE XI-2-continued

ANTIBIOTICS***	ORIGINAL SMALL PLATES			IMPROVED SMALL PLATES		
	CFU	% RECOVERY	S FACTOR	CFU	% RECOVERY	S FACTOR
Gentamicin (6)	+	+	+	630	92	.7 ± .2
Tobramycin (4)	+	+	+	+	+	+
Amikacin (21)	+	+	+	+	+	+
Penicillin (20)	+	+	+	+	+	+
Ampicillin (21)	+	+	+	+	+	+
Cephalothin (20)	214	100	.4 ± .2	777	100	.8 ± .1
Cefoxitin (25)	467	96	.7 ± .2	301	99.8	.9 ± .1
Chloramphenicol (18)	+	+	+	+	+	+
Tetracycline (9)	266	100	.3 ± .3	581	99	.8 ± .1
Erythromycin (8)	512	100	.9 ± .2	375	100	.6 ± .2
Gantrisin (100)	+	+	+	+	+	+
Clindamycin (5)	+	+	+	+	+	+
Methicillin (9)	+	+	+	+	+	+
Vancomycin (8)	+	+	+	+	+	+
Piperacillin (60)	+	+	+	+	+	+
Moxalactam (100)	+	+	+	+	+	+
Cefotaxime (20)	564	100	.8 ± .1	567	100	.9 ± .1
II. <i>Escherichia coli</i> (ATCC #29522)						
No Drug	237	91	1.3 ± .3	172	94	1.5 ± .6
Gentamicin (6)	+	+	+	+	+	+
Tobramycin (4)	+	+	+	+	+	+
Amikacin (21)	+	+	+	+	+	+
Penicillin (20)	+	+	+	+	+	+
Ampicillin (21)	+	+	+	+	+	+
Cephalothin (20)	102	100	.1 ± .09	288	92	.8 ± .1
Cefoxitin (25)	454	100	.5 ± .1	406	99	.9 ± .3
Chloramphenicol (18)	217	99.5	1.0 ± .3	420	96	1.1 ± .4
Tetracycline (9)	+	+	+	+	+	+
Erythromycin (8)	305	100	.9 ± .2	212	99	1.0 ± .2
Gantrisin (100)	140	99.8	1.4 ± .6	231	99	1.0 ± .1
Ticarcillin (150)	+	+	+	+	+	+
Carbenicillin (20)	+	+	+	+	+	+
Piperacillin (60)	+	+	+	+	+	+
Cefamandole (20)	167	99	.5 ± .2	209	98	.8 ± .1
Kanamycin (14)	330	100	.05 ± .03	352	99.6	.5 ± .2

All tubes were held at room temperature (20 C.) for two hours prior to processing.

The large plates contained 80 ml of agar media.

***Numbers in parenthesis represent final concentration of antibiotic used in microorganisms/ml of blood.

+ Not tested because recovery is good on small plates.

TABLE XI-3

	ENTEROBACTER CLOACAE			
	% Recovery		S-Factor	
	Old	New	Old	New
#1344-2 - SMALL PLATES				
Ampicillin	99.5	95	1.5 ± .5	.8 ± .1
Carbenicillin	0	94	0	.6 ± .3
Ticarcillin	100	86	.06 ± .07	1.3 ± .6
Tobramycin	100	75	.03 ± .01	.9 ± .2
Chloramphenicol	98	80	.9 ± .2	1.2 ± .3
Tetracycline	98	88	.9 ± .5	1.4 ± .7
Gantrisin	97	99	.6 ± .1	.8 ± .1
No Drug	97	98	1.4 ± .2	1.2 ± .1
Cefoxitin	95	97	.9 ± .2	.8 ± .2
Cephalothin	99.6	88	1.0 ± .2	.9 ± .1
Gentamicin	95	99	.04 ± .03	1.1 ± .1
LARGE PLATES				
Tetracycline	97	96	.7 ± .1	.9
Tobramycin	88	93	.9 ± .5	1.1
Chloramphenicol	+	97	+	.9

+ Not tested because recovery is good on small plates.

TABLE XI-4

	KLEBSIELLA PNEUMONIAE			
	% Recovery		S-Factor	
	Old	New	Old	New
#632-2 - SMALL PLATES				
Ampicillin	97	93	.5 ± .3	1.0 ± 1
Carbenicillin	93	94	.1 ± .1	.8 ± .2
Ticarcillin	99	89	1.0 ± .1	.9 ± .1

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TABLE XI-4-continued

	KLEBSIELLA PNEUMONIAE			
	% Recovery		S-Factor	
	Old	New	Old	New
LARGE PLATES				
Carbenicillin	92	88	.5 ± .3	.7

55

TABLE XI-5

	PSEUDOMONAS AERUGINOSA			
	#27853 - SMALL PLATES		S-Factor	
	Old	New	Old	New
#632-2 - SMALL PLATES				
Ampicillin	97	94	.6 ± .4	.8 ± .2
Carbenicillin	98	95	.9 ± .3	.9 ± .1
Ticarcillin	93	91	.3 ± .1	1.2 ± .1
Tobramycin	98	90	1.0 ± .1	.9 ± .2
Chloramphenicol	96	86	.7 ± .2	1.1 ± .2
Tetracycline	95	89	1.0 ± .1	1.2 ± .1
Gantrisin	99	98	1.2 ± .2	.9 ± .1
No Drug	97	97	1.6 ± .3	.9 ± .2
Cefotaxime	99	96	.9 ± .2	1.1 ± .3
Cefoxitin	97	86	1.4 ± .2	.9 ± .4

60

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TABLE XI-5-continued

<i>PSEUDOMONAS AERUGINOSA</i> #27853 - SMALL PLATES							
% Recovery		S-Factor					
Old	New	Old	New				
Cephalothin	90	92	1.4 ± .1	1.4 ± .01			
Gentamicin	98	56	1.0 ± .4	1.2 ± .2			
Moxalactam	97	87	.6 ± .1	.9 ± .1			

TABLE XI-6

<i>STREPTOCOCCUS PNEUMONIAE</i>							
% Recovery		S-Factor					
Old	New	Old	New				
#6301 - SMALL PLATES							
Penicillin	76	63	.02 ± .16	.8 ± .2			
Ampicillin	43	65	.01 ± .01	.6 ± .2			
Methicillin	83	86	.003 ± .004	.4 ± .2			
Tobramycin	97	88	.8 ± .4	.6 ± .4			
Chloramphenicol	99	93	.6 ± .4	.8 ± .2			
Tetracycline	100	99	.3 ± .2	.4 ± .2			
Erythromycin*	97	98	.3 ± .3	1.3 ± .3			
Cefoxitin	97	99	.4 ± .1	.5 ± .2			
No Drug	93	90	1.0 ± .03	1.0 ± .1			
Gentamicin	99.8	100	1.0 ± .1	1.1 ± .1			
LARGE PLATES							
Tetracycline	99	100	.5 ± .2	.7 ± .2			
Tobramycin	98	99	1.0 ± .3	1.1 ± .2			
Ampicillin	+	82	+	.9 ± .3			
Cefoxitin	100	99	.2 ± .1	.8 ± .1			
Methicillin	+	97	+	.6 ± .1			
Penicillin	+	63	+	.9 ± .1			

*Incubation period - 48 hours.

+ Not tested because recovery is good on small plates.

TABLE XI-7

<i>STREPTOCOCCUS PYOGENES</i>							
% Recovery		S-Factor					
Old	New	Old	New				
#19615 - SMALL PLATES							
Penicillin	0.2	100	.02 ± .02	.6 ± .2			
Ampicillin	0	99	.0002 ± .000	3.6 ± .1			
Methicillin	95	90	.2 ± .1	.8 ± .2			
Tobramycin	98	100	.6 ± .1	.5 ± .2			
Chloramphenicol	98	97	.5 ± .1	.4 ± .2			
Tetracycline	100	96	.3 ± .1	.1 ± .1			
Erythromycin	100	100	.02 ± .01	.02 ± .01			
Cefoxitin	98	100	.3 ± .1	.2 ± .03			
No Drug	92	95	1.0 ± .5	.5 ± .1			
Gentamicin	99	94	.6 ± .1	.9 ± .1			
LARGE PLATES							
Methicillin	99	99.8	.6 ± .1	1.7 ± .2			
Tobramycin	99	100	1.0 ± .1	1.1 ± .3			
Chloramphenicol	99	99	.8 ± .3	.9 ± .3			
Tetracycline	99.6	100	.6 ± .1	.7 ± .3			
Erythromycin	100	100	.1 ± .1	.1 ± .1			
Cefoxitin	98	95	.6 ± .1	.7 ± .2			
No Drug	98	97	.8 ± .1	1.0 ± .2			
Ampicillin	+	100	+	1.5 ± .2			
Gentamicin	+	99.5	+	1.2 ± .3			

TABLE XI-8

<i>HAEMOPHILUS INFLUENZAE</i>							
% Recovery		S-Factor					
Old	New	Old	New				
#19418 - SMALL PLATES							
No Drug	89	78	.6 ± .2	.9 ± .4			
Ampicillin	33	95	.01 ± .01	.9 ± .1			
Cefoxitin	80	97	.1 ± .1	.7 ± .2			
Clindamycin	96	99	1.2 ± .2	1.1 ± .2			
Erythromycin	94	100	.4 ± .1	.7 ± .2			
Gentamicin	90	77	.4 ± .1	1.3 ± .4			
Kanamycin	94	99	.8 ± .1	.9 ± .1			

TABLE XI-8-continued

<i>HAEMOPHILUS INFLUENZAE</i>							
% Recovery		S-Factor					
Old	New	Old	New				
Methicillin	94	99	.9 ± .2	.8 ± .1			
Penicillin	73	99	.1 ± .1	.6 ± .1			
Tetracycline	100	99	.2 ± .1	.6 ± .1			
Vancomycin	95	99	.7 ± .2	.8 ± .2			

<i>LARGE PLATES</i>							
% Recovery		S-Factor					
Old	New	Old	New				
No Drug	95	99	.9 ± .1	1.2 ± .1			
Cefoxitin	93	95	.8 ± .6	.7 ± .2			
Gantrisin	92	98	1.2 ± .7	.6 ± .1			
Penicillin	100	99	.3 ± .2	.6 ± .2			

<i>BACTEROIDES FRAGILIS</i>							
% Recovery		S-Factor					
Old	New	Old	New				
#23745 - SMALL PLATES							
No Drug	88	51	.7 ± .2	1.0 ± .3			
Carbenicillin	96	82	.09 ± .05	.5 ± .1			
Cefotaxime	97	100	.7 ± .2	.8 ± .1			
Cefoxitin	94	99	.5 ± .3	1.1 ± .5			
Chloramphenicol	87	88	.9 ± .2	.9 ± .1			
Erythromycin	98	64	.7 ± .5	.6 ± .2			
Penicillin	87	51	.7 ± .1	.7 ± .1			
Tetracycline	90	90	.5 ± .1	.5 ± .1			
Vancomycin	95	99	.7 ± .2	.8 ± .2			

TABLE XI-10

<i>CLOSTRIDIUM SPOROGENES</i>							
% Recovery		S-Factor					
Old	New	Old	New				
#19404 - SMALL PLATES							
No Drug	97	93	.6 ± .1	.6 ± .2			
Carbenicillin	98	99	.3 ± .3	.8 ± .3			
Cefotaxime	97	98	.5 ± .2	.5 ± .2			
Chloramphenicol	96	97	.7 ± .4	.6 ± .4			
Clindamycin	99.7	100	.4 ± .2	.3 ± .2			
Erythromycin	96	99	.5 ± .2	.8 ± .2			
Gantrisin	93	99	.5 ± .1	.7 ± .1			
Gentamicin	98	98	.8 ± .2	.6 ± .4			
Penicillin	100	96	.08 ± .04	.8 ± .3			

Once again a new cocktail protected the microorganisms from the cidal effect of the antibiotics. As expected for those antibiotics which do not exert a cidal effect, both the original tube and the modified tube containing the specimen transport system yielded the same actual recovery of microorganisms. A large dilution is apparently needed (1:267) when dealing with a few specific organism-antibiotic combinations, e.g., *S. aureus* with cephalothin. These data suggest that large dilutions will only be required for a few antibiotics, e.g., cephalothin, tetracycline, erythromycin, and certain organisms, e.g., + cocci. The aminoglycosides, penicillin, ampicillin, and chloramphenicol are completely neutralized by the cocktail while the cephalothins are partially neutralized.

EXAMPLE XII

A series of the original tubes as described in Example XI and the tubes containing the specimen transport system as described in Example XI were utilized to process blood from patients suspected of having septicemia with confirmed positive blood cultures. In each case, blood from the patient was placed in the original tube and the modified tube containing the specimen

transport system. The tubes were centrifuged and the concentrated residue plated on the small petri plates described in Example XI. The results of the tests are set forth in Table XII-1 below.

TABLE XII-1

CULTURE NO.	ORGANISM	COUNT/ML			ANTIBIOTIC IN SERUM AT TIME OF DRAW
		ORIGINAL SYSTEM	NEW SYSTEM	% OF CHANGE	
1.	<i>Acinetobacter</i> sp.	NG*	1.4	—	None
2.	<i>Enterobacter agglomerans</i>	0.7	0.7	0	None
3.	<i>Enterobacter cloacae</i>	0.1	NG	—	Tobramycin & Cefazolin
4.	<i>Enterobacter cloacae</i>	NG	0.1	—	Cefoxitin
5.	<i>Escherichia coli</i>	0.6	2.9	+383	Penicillin & Tobramycin
6.	<i>Escherichia coli</i>	1.1	.7	-57	Penicillin & Tobramycin
7.	<i>Escherichia coli</i>	13.0	92.8	+614	None
8.	<i>Escherichia coli</i>	7.3	12.5	+71	Ticarcillin & Gentamicin
9.	<i>Escherichia coli</i>	2.1	10.2	+385	None
10.	<i>Escherichia coli</i>	NG	0.1	—	Mefoxin
11.	<i>Escherichia coli</i>	NG	0.1	—	Penicillin & Tobramycin
12.	<i>Flavobacterium</i>	NG	0.6	—	—
13.	<i>Histoplasma capsulatum</i>	10.5	7.0	-50	Amphotericin B
14.	<i>Histoplasma capsulatum</i>	1.6	2.6	+62	Amphotericin B
15.	<i>Histoplasma capsulatum</i>	13.5	14.2	+05	Amphotericin B
16.	<i>Klebsiella oxytoca</i>	8.8	20.6	+134	None
17.	<i>Klebsiella oxytoca</i>	2.1	3.9	+86	Gentamicin & Ticarcillin
18.	<i>Klebsiella pneumoniae</i>	0.1	0.1	—	Gentamicin
19.	<i>Klebsiella pneumoniae</i>	11.9	19.3	+62	Tobramycin & Carbenicillin
20.	<i>Klebsiella pneumoniae</i>	130.8	78.1	-67	None
21.	<i>Klebsiella pneumoniae</i>	163.0	182.0	+12	Tobramycin & Carbenicillin
22.	<i>Klebsiella pneumoniae</i>	0.3	NG	—	Tobramycin & Carbenicillin
23.	<i>Klebsiella pneumoniae</i>	0.3	1.0	+227	Gentamycin & Ticarcillin
24.	<i>Klebsiella pneumoniae</i>	7.3	8.4	+15	Gentamycin & Ticarcillin
25.	<i>Listeria monocytogenes</i>	0.4	9.5	+227	Cefoxitin
26.	<i>Pseudomonas aeruginosa</i>	131.3	83.0	-58	Tobramycin & Carbenicillin
27.	<i>Pseudomonas fluorescens</i>	0.1	NG	—	None
28.	<i>Staphylococcus aureus</i>	5.6	12.2	+118	Methicillin

*No growth

CONCLUSIONS:

1. In 68% of the positive samples the new tube yielded more organisms/ml of blood. The difference ranged between a low of 5% and a high of 614% increased count.
 2. The new system missed three positives while the original system missed five.
 3. In four cases the original system gave a higher count. However, this level is well within expected experimental variability.
 4. Thirty-six percent (36%) of the patients were not on antibiotics at the time of blood collection. The new system yielded higher counts in 50% of the cases. The difference ranged from a low of 134% and a high of 614% increase.
 5. Seventeen (17) cultures were simultaneously positive at the same time. Two cultures (one *Listeria* and one *Escherichia coli*) were positive one day earlier in the new system.

As shown in the table, in 68% of the samples, the modified device containing the specimen transport system yielded higher counts (which ranged between 5% and 614% increase) than did the original device. In five instances, the original device was negative and the new device positive. Although the majority of samples were positive at the same time, there were two cases in which the new device detected a positive culture one day earlier (*E. coli* and one *Listeria* specimen). Surprisingly, the new device appears to yield greater counts even when the patient was not on antibiotics (3 patients). This indicates that the new device containing the specimen transport system more effectively blocked the patient's immune system than did the liquid blood treating solution of the original device.

EXAMPLE XIII

A first series of original lysis-centrifugation devices were assembled as described in Example VII. A second series of lysis-centrifugation devices were assembled the same as the second series of such devices in Example 5.

A third series of lysis-centrifugation devices were assembled as follows:

To the article as disclosed in U.S. Pat. No., 4,212,948 were added 0.3 milliliters of FLUORINERT FC48 as

% OF CHANGE	ANTIBIOTIC IN SERUM AT TIME OF DRAW
—	None
0	None
—	Tobramycin & Cefazolin
—	Cefoxitin
+383	Penicillin & Tobramycin
-57	Penicillin & Tobramycin
+614	None
+71	Ticarcillin & Gentamicin
+385	None
—	Mefoxin
—	Penicillin & Tobramycin
—	—
-50	Amphotericin B
+62	Amphotericin B
+05	Amphotericin B
+134	None
+86	Gentamicin & Ticarcillin
—	Gentamicin
+62	Tobramycin & Carbenicillin
-67	None
+12	Tobramycin & Carbenicillin
—	Tobramycin & Carbenicillin
+227	Gentamycin & Ticarcillin
+15	Gentamycin & Ticarcillin
+227	Cefoxitin
-58	Tobramycin & Carbenicillin
—	None
+118	Methicillin

cushioning agent along with the following compounds in dry particulate powder form:

- 0.008 grams of thioglycolate;
- 0.048 grams of sodium polyanetholsulfonate; and
- 0.018 grams of purified saponin.

The tubes in the third series were evacuated sufficient to draw 8 milliliters of blood. This series of tubes was then heated to 121° C. for 30 minutes and then allowed to cool to room temperature.

50 In each instance, the stated amount of specific micro-
organisms and antibiotics (if any) as illustrated in Tables
XII-1 through XII-6 below was added to 7.5 milliliters
of blood in the first and second series of tubes and 8
milliliters of blood in the third series of tubes. The blood
55 was then deposited into the respective lysis-centrifuga-
tion tube and each tube was subjected to centrifugation
as described in this specification. Like quantities of each
microbial pathogen-antibiotic combination were plated
on both large and small petri plates as described in
60 Example XI. The results are set forth in Tables XIII-1
through XIII-6 below:

TABLE XIII-1

	<i>Staphylococcus aureus</i>							
	ORIGINAL				NEW LIQUID			
Antibiotics	Small Plate		Large Plate		Small Plate		Large Plate	
	% Recovery	S-Factor	% Recovery	S-Factor	% Recovery	S-Factor	% Recovery	S-Factor

TABLE XIII-1-continued

<i>Staphylococcus aureus</i>							
No Drug	91	.88	*	*	95	1.06	*
Ampicillin	84	.033	NT	NT	99	1.10	NT
Cefamandole	100	.006	100	.099	99	.12	100
Erythromycin	100	.06	100	.85	83	.068	100
Vancomycin	98	.58	*	*	98	.75	*

NEW POWDER							
Antibiotics	Small Plate		Large Plate		% Recovery	S-Factor	S-Factor
	% Recovery	S-Factor	% Recovery	S-Factor			
No Drug	95	.95	*	*			
Ampicillin	94	.96	NT	NT			
Cefamandole	50	.03	99	.31			
Erythromycin	72	.80	*	*			
Vancomycin	52	.76	*	*			

*Unnecessary to test large plates

NT = Not tested

TABLE XIII-2

Escherichia coli										
Antibiotics	ORIGINAL				NEW LIQUID				S-Factor	
	Small Plate		Large Plate		Small Plate		Large Plate			
	% Recovery	S-Factor								
No Drug	98	1.17	*	*	98	.78	94	1.5		
Cephalothin	100	.04	100	.10	99	.07	100	.36		
Moxalactam	38	.021	75	.008	100	.10	*	*		
NEW POWDER										
Antibiotics	Small Plate				Large Plate				S-Factor	
	% Recovery	S-Factor								
No Drug	93	1.10	*	*	95	.76	92	.83		
Cephalothin	95	.76	92	.83	100	.017	100	.40		
Moxalactam	100	.017	100	.40						

*Unnecessary to test large plates

TABLE XIII-3

TABLE III										
Streptococcus pneumoniae										
Antibiotics	ORIGINAL				NEW LIQUID				S-Factor	
	Small Plate		Large Plate		Small Plate		Large Plate			
	% Recovery	S-Factor	% Recovery	S-Factor	% Recovery	S-Factor	% Recovery	S-Factor		
No Drug	92	.70	94	.81	84	.76	95	1.00		
Ampicillin	43	.0084	NT	NT	81	.52	95	.25		
Cefoxitin	97	.39	100	.16	99	.51	100	.27		
Penicillin	76	.024	NT	NT	87	.57	93	.43		
NEW POWDER										
Antibiotics		Small Plate		Large Plate						
		% Recovery	S-Factor	% Recovery	S-Factor	% Recovery	S-Factor	% Recovery	S-Factor	
No Drug		96	.88	69	.97					
Ampicillin		65	.60	85	.88					
Cefoxitin		99	.28	99	.77					
Penicillin		63	.78	63	.87					

NT = Not issued

TABLE XIII-4

Enterobacter Cloacae										
Antibiotics	ORIGINAL				NEW LIQUID				NEW POWDER	
	Small Plate		Large Plate		Small Plate		Large Plate			
	% Recovery	S-Factor								
No Drug	97	1.42	*	*	—	—	*	*		
Chloramphenicol	98	.91	*	*	89	1.6	97	.93		
Tobramycin	100	.028	98	.94	94	.70	93	1.07		

TABLE XIII-4-continued

		Enterobacter Cloacae		Small Plate		Large Plate	
		% Recovery	S-Factor	% Recovery	S-Factor		
	No Drug	98	1.20	*	*		
	Chloramphenicol	80	1.15	*	*		
	Tobramycin	75	.85	*	*		

*Unnecessary to test large plates

— Discontinued production of liquid tube (dry results only)

TABLE XIII-5

<i>Pseudomonas aeruginosa</i>								
ORIGINAL				NEW LIQUID				
Antibiotics	Small Plate		Large Plate		Small Plate		Large Plate	
	Recovery	S-Factor	Recovery	S-Factor	Recovery	S-Factor	Recovery	S-Factor
Ampicillin	97	.56	*	*	87	.84	*	*
Barbenicillin	97	.125	*	*	92	.85	*	*
NEW POWDER								
Antibiotics		Recovery	S-Factor	Recovery	S-Factor	Recovery	S-Factor	
Ampicillin		94	.83	*	*	*	*	
Barbenicillin		56	.92	*	*	*	*	

*Unnecessary to test large plates

TABLE XIII-6

<i>Klebsiella pneumoniae</i>								
ORIGINAL				NEW LIQUID				
Antibiotics	Small Plate		Large Plate		Small Plate		Large Plate	
	Recovery	S-Factor	Recovery	S-Factor	Recovery	S-Factor	Recovery	S-Factor
Barbenicillin	93	.143	92	.54	93	.66	88	.72
Cefoxitin	49	.02	NT	NT	72	.86	NT	NT
NEW POWDER								
Antibiotics		Recovery	S-Factor	Recovery	S-Factor	Recovery	S-Factor	
Barbenicillin		94	.76	*	*	*	*	
Cefoxitin		97	.98	NT	NT	NT	NT	

*Unnecessary to test large plates

NT = Not tested

As can be seen from the data above, the lysis-centrifugation devices made in accordance with the subject invention that contain the dry particulate powdered specimen transport system of the subject invention performed at least as well as the systems in accordance with this invention containing the specimen transport system in aqueous solution within the tube. Both of the new systems clearly out perform the original system as set forth in the Examples.

EXAMPLE XIV

Increasing Hold Time for Blood Specimens

A first series of original lysis-centrifugation devices were assembled as described in Example VII. A second series of lysis-centrifugation devices were assembled in the same manner as the third series of lysis-centrifugation devices which were used to obtain the data set forth in Tables XIII-1 through XIII-6 of Example XIII.

In each instance, the stated amount of specific micro-organism as illustrated in Table XIV-1 below was added to 7.5 ml. of blood in the first series of tubes and to 8 ml. of blood in the second series of tubes. The blood was then deposited in the respective lysis-centrifugation

tubes and the tubes were then held at 21° C. for the time period set forth in Table XIV-1 below. Each tube was next subjected to centrifugation and the concentrated 50 contents plated on growth media as described in the specification.

As can be seen from the data set forth in Table XIV-1, certain species of bacteria propagate or die in the original tube when held for a period of 24 hours. Surprisingly, these same species did not substantially grow or die in the second series of new tubes containing the specimen transport system. While it is not recommended that the centrifugation tubes be held for lengthy periods of time, it has been found in the hospital environment that such tubes are held for time periods before processing. While the data shows no substantial propagation of most species within the new tube at 24 hours, it is believed that the tubes should be processed as quickly as possible and certainly before a hold time of 12 hours has been completed. Furthermore, to assure against growth of some species of bacteria such as *Enterobacter cloacae*, sodium chloride can be added, such as in the urine examples as set forth in Example XVII

below. Sodium chloride can be present in an amount from about 0.1% to about 10% by weight of the final process treating solution and blood sample, and preferably in the range of from about 1% to about 5% and most preferably at about 3%.

TABLE XIV-1

Organism		CFU	% Recovery		S-Factor
			2 HR	24 HR	2 HR
<i>Haemophilus influenzae</i>	original	501	86	91	.8 ± .2
(19418)	new	501	93	76	.8 ± .1
<i>Streptococcus pyogenes</i>	original	142	99.7	89	.8 ± .3
(1344-2)	new	142	100	100	.9 ± .3
<i>Pseudomonas aeruginosa</i>	original	332	99	TNTC	2.2 ± .8
(27853)	new	332	95	94	1.2 ± .44
<i>Staphylococcus aureus</i>	original	552	99	100	.8 ± .03
(25923)	new	1317	98	97	1.0 ± .3
<i>Escherichia coli</i>	original	826	98	TNTC	1.7 ± .5
(25922)	new	1057	100	100	.9 ± .11
<i>Streptococcus pneumoniae</i>	original	602	93	98	1.0 ± .3
(6301)	new	360	90	99	1.0 ± .1
<i>Enterobacter cloacae</i>	original	1236	98	—	1.2 ± .5
(1344-2)	new	1236	99	—	.9 ± .2

TNTC = too numerous to count

EXAMPLE XV

Use of an Enzyme Component

A series of lysis-centrifugation devices were assembled the same as the second series of devices containing the specimen transport system as in Example XIV. To each tube was added 8 ml. of blood containing 842 CFU of *E. coli* and 20 ug/ml. of the antibiotic cefotaxime as well as the stated amount of beta-lactamase enzyme illustrated in Table XV-1 below. The beta-lactamase enzyme used was beta-lactamase (*Bacillus cereus*), lot No. 203435, order No. 426205, Calbiochem-Behring Corporation, La Jolla, California, NOTE: beta-lactamase I - 13 units of activity to beta-lactamase II - 1 unit of activity. The blood was then deposited into the respective lysis-centrifugation tubes and each tube was subjected to centrifugation as described in the specification. Like quantities of microbial pathogen-antibiotic-enzyme combination were plated on small petri plates as described in Example XI. The results are set forth in Table XV-1 below.

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TABLE XV-1

<i>E. coli</i> - Cefotaxime 20 ug/ml.		
Units Of Enzyme	Percent Recovery	S-Factor
0	58	.02
.01	100	.011
0.1	100	.052
1.0	99	.33
2.0	99	1.17
4.0	99	.82
5.0	99.8	.95

As can be seen from Table XV-1 the beta-lactamase as an integral part of the specimen transport system will effectively function to block the activity of the antibiotic and prevent killing of the microbial pathogen while contained within the lysis-centrifugation tube.

As can be seen from Table XV-1 the beta-lactamase as an integral part of the specimen transport system will effectively function to block the activity of the antibiotic and prevent killing of the microbial pathogen while contained within the lysis-centrifugation tube.

As a comparison, a second series of tubes were assembled as described in Example 1 and to each tube was added 765 CFU of *E. coli*, 20 ug/ml. of cefotaxime, the units of beta-lactamase enzyme as illustrated in Table XV-2 and 7.5 ml. of blood. The tubes were then centrifuged and samples were cultured as described above, and the results are set forth in Table XV-2 below.

TABLE XV-2

Units Of Enzyme	Percent Recovery	S-Factor
0	93	.08
1	90	.04
5	95	.155

The results of Table XV-2 when compared with Table XV-1 indicate that the addition of the enzyme does not satisfactorily improve the S values when used in a lysis-centrifugation tube which does not contain the specimen transport system.

Further tests were made comparing a first series of lysis-centrifugation tubes identical to those prepared in conjunction with Table XV-2 above and containing no specimen transport system; a second series of lysis-centrifugation tubes identical to those used in conjunction with Table XV-1 above but containing no enzyme; and a third series of lysis-centrifugation tubes which were the same as the second series of tubes but which contained the indicated amounts of beta-lactamase enzyme as set forth in Tables XV-3 through XV-8 below. The blood containing between 200 and 1000 CFU of the indicated bacteria was added to each tube and the tubes were processed as described above in this example and the results are set forth in Tables XV-3 through XV-8 below:

TABLE XV-3

Antibiotic	ug/ml	First Series of Tubes	Second Series of Tubes	Third series of Tubes With Enzyme Units								
				No Enzyme	.01	.1	1.	2.	2.5	3.	4.	5.
Cephalothin	20	.04	.05				.40	.90				1.30
Cefamandole	20	.35	.35				1.0	.63				
Cefoxitin	25	.27	.76				.91				1.76	

TABLE XV-3-continued

Antibiotic	ug/ml	of Tubes	First Series		Second Series		Third series of Tubes With Enzyme Units						
					No Enzyme	.01	.1	1.	2.	2.5	3.	4.	5.
Cefotaxime	20	.08			.02	.01	.05	.33	1.2		.82	.95	
Moxalactam	100	.02			.01			.03	.05		.004	.04	.002
Moxalactam	50				.03			.04			.05		
Moxalactam	40				.01			.10	.22		.12	.16	
Moxalactam	20				.33			.20			.16	.65	
Moxalactam	10				.84			1.01			.92		
Cefob		.001			.003			.84			1.01		

TABLE XV-4

Antibiotic	ug/ml	of Tubes	First Series		Second Series		Third Series of Tubes With Enzyme Units						
					No Enzyme	.01	.1	1.	2.	2.5	3.	4.	5.
Cephalothin	20	.08			.04	.03	.002	.50	.85				1.65
Cefamandole	20	.006			.03			.81			.62		.81
Cefotaxime	20	.28			.52			.96			1.02		
Cefob	50	.009			.025			.72			.80		

STS = Specimen Transport System

TABLE XV-5

Antibiotic	ug/ml	of Tubes	First Series		Second Series		Third Series of Tubes With Enzyme Units						
					No Enzyme	.01	.1	1.	2.	2.5	3.	4.	5.
Cefotaxime	20	0			0			.03			.58		
Moxalactam	20	—			—			.23			.22		

STS = Specimen Transport System

TABLE XV-6

Antibiotic	ug/ml	of Tubes	First Series		Second Series		Third Series of Tubes With Enzyme Units						
					No Enzyme	.01	.1	1.	2.	2.5	3.	4.	5.
Cefotaxime	20	.12			.013			.79			.52		
Moxalactam	20	—			—			.12			.26		

STS = Specimen Transport System

TABLE XV-7

Antibiotic	ug/ml	of Tubes	First Series		Second Series		Third Series of Tubes With Enzyme Units						
					No Enzyme	.01	.1	1.	2.	2.5	3.	4.	5.
Cefotaxime	20	.003			.001			.153			.46		
Moxalactam	40	—			—			.005			.004		

TABLE XV-8

Antibiotic	ug/ml	of Tubes	First Series		Second Series		Third Series of Tubes With Enzyme Units						
					No Enzyme	.01	.1	1.	2.	2.5	3.	4.	5.
Cefoxitin	25	.39			.28			.33			.18		
Cefotaxime	20	.001			0			.23			.62		

STS = Specimen Transport System

The data shown in Tables XV-3-XV-8 show that the addition of the enzyme to the specimen transport factor system of the subject invention effectively enhances the

neutralization properties thereof for the above indicated class of antibiotics.

EXAMPLE XVI

Sterilization of Enzyme-Containing Specimen Transport System in a Specialized Apparatus

Lysis-centrifugation tubes containing the antibiotic deactivation system utilized in the second series as set forth in Tables XV-3 through XV-8 above were made up. To a first series of these tubes was added the amount of beta-lactamase enzyme set forth in Table XVI-1 below. The tubes were then subjected to cobalt sterilization and thereafter 8 ml. of blood containing the microbial pathogen and the antibiotic as set forth in Table XVI-1 were added thereto and processed as set forth in Example XV. A second series of the tubes were steam sterilized and thereafter the indicated amount of beta-lactamase enzyme was added thereto and thereafter the 8 ml. of blood with the indicated amount of microbial pathogen and antibiotic was added thereto and the tubes were centrifuged and processed as set forth in Example XV. The results of these tests were set forth in Tables XVI-1 and XVI-3 below.

TABLE XVI-1

COBALT STERILIZATION					
	<i>E. coli</i> 25922 cefotaxime 20 μ g/ml		<i>Staph. aureus</i> 25923 cephalothin 20 μ g/ml		
Units of Enzyme	Percent Recovery	S-Factor	Percent Recovery	S-Factor	
0.1	67	.01	100	.04	
1.0	83	.06	97	.17	
5.0	81	.41	97	.33	

TABLE XVI-2

STEAM AUTOCLAVE STERILIZATION					
	<i>E. coli</i> 25922 cefotaxime 20 μ g/ml		<i>Staph. aureus</i> 25923 cephalothin 20 μ g/ml		
Units of Enzyme	Percent Recovery	S-Factor	Percent Recovery	S-Factor	
0.1	100	.05	17	.002	
1.0	99	.33	26	.50	
5.0	99.8	.95	34	1.65	

As can be seen by a comparison of the data in Table XVI-1 with Table XVI-2, the loss of enzyme activity due to cobalt sterilization ranges from 20 % to 80%, depending on the concentration of the enzyme. However, Table XVI-1 clearly illustrates that cobalt sterilization can be effectively utilized, and when used, increased amounts of the enzyme should be added to the tube prior to the sterilization. It should be noted that other chemicals are anticipated for use within the specimen transport system depending somewhat on the type of antimicrobial factors which are anticipated to be present in the sample. For example, other water-soluble compounds which are antagonistic to other classes of antimicrobial substances such as sodium hypochlorite, heavy metals and the like include substances like sodium bisulfite and sulphydryls, for example. As indicated, the specimen transport system of the subject invention finds special utility in the lysis-centrifugation tube such as set forth in U.S. Pat. No. 4,131,512 and U.S. Pat. No. 4,212,948. In addition, the specimen transport system finds utility in the lysis-centrifugation tube as set forth in U.S. Pat. No. 4,164,449. In addition, the specimen transport system of the subject invention can be utilized in a blood treating tube for neonates which simply will include a standard single stopper vacuum tube designed to draw between 1 and 2 milliliters of

blood. The tube would contain no substance other than the specimen transport system of the subject invention and saponin if desired. The blood can be treated upon injection in the tube and then directly plated upon growth media.

The above examples illustrate the beneficial effect of the specimen transport system of the subject invention when used in a lysis-centrifugation tube for analyzing microbial pathogens within blood samples. However, the specimen transport system of the present invention finds utility in protecting microorganisms in sample fluids other than blood which are collected and later analyzed for the presence of microbial pathogens. For example, the specimen transport system of the subject invention can protect microorganisms present in swabs, urine, sputum, spinal fluid and other body fluids during transit. It is well known that these fluids also contain both humoral and chemical antimicrobials (if the patient is being treated with antibiotics). With urine samples, the concentration of antibiotics may actually exceed that present in serum. An example of a modified specimen transport system for neutralizing antibiotics in urine is present in Example XVII below.

EXAMPLE XVII

Maintaining the Microbial Integrity of a Urine Specimen

The following example was performed to test the ability of the specimen transport system urine cocktail to block conventional therapeutic antibiotics and hold the microbial population, present in the urine, stable for up to 24 hours.

The following dry mixture was placed in each of a series of tubes:

- 0.03 grams of sodium polyanethosulfonate
- 0.005 grams of thioglycolate
- 0.1 grams of ICN free-base cysteine
- 0.1 grams of sodium bicarbonate.

The various antibiotics, listed in Tables XVII-1-XVII-6 below, were added, at the concentrations also specified therein, to the tubes containing the above-described specimen transport system urine cocktail and to an equal number of tubes without the urine cocktail. Five milliliters of sterile urine was then added to all tubes after which the tubes were vigorously mixed. Control tubes contained either urine alone or urine plus the above-described specimen transport system urine cocktail. No antibiotics were added to control tubes. The microorganisms listed in Tables XVII-1-XVII-6 below were adjusted to a McFarlin of 0.5 and then diluted 1:100 with sterile culture media. A 0.1 milliliter aliquot of a single microorganism was added to each urine containing tube and the resultant mixture vigorously agitated. A ten microliter aliquot from each tube containing the resultant mixture was immediately inoculated on tryptic soy agar plates and spread with a sterile spreader. The inoculated plates were incubated overnight in an environment and temperature appropriate for the microorganism employed. The tubes were then allowed to stand at room temperature for 24 hours. Additional ten microliter aliquots were plated as before herein described at the time intervals indicated in Tables XVII-1-XVII-6 below. All plating was done in quadruplicate and the S-Factor recorded as an average of the quadruplicate plating in Tables XVII-1-XVII-6 below.

TABLE XVII-1

Antibiotic**	Hour Time Points			
	2	4	6	24
*No Drug	—	.92	1.05	.84
No Drug	—	2.01	TNTC	TNTC
*Amikacin (210)	—	.73	.62	.33
Amikacin (210)	—	0	0	0
*Ampicillin (210)	—	.92	.75	.70
Ampicillin (210)	—	.02	.001	0
*Carbenicillin (200)	1.12	.90	—	.31
Carbenicillin (200)	.83	.37	—	.003
*Cefamandole (200)	.88	.45	—	.31
Cefamandole (200)	.37	.33	—	.005
*Cefobid (500)	—	1.16	1.63	1.68
Cefobid (500)	—	.03	0	0
*Cefotaxime (200)	—	1.03	1.55	1.65
Cefotaxime (200)	—	.009	0	0
*Cefoxitin (250)	.72	.33	—	.17
Cefoxitin (250)	.23	.05	—	.002
*Cephalothin (200)	—	.47	.86	.53
Cephalothin (200)	—	.01	.01	.04
*Chloramphenicol (180)	—	1.03	1.03	.89
Chloramphenicol (180)	—	0	0	0
*Erythromycin (80)	—	.70	.75	.59
Erythromycin (80)	—	1.36	1.49	.42
*Gantrisin (1000)	.70	.93	—	.56
Gantrisin (1000)	0	0	—	0
*Gentamicin (60)	—	.81	.38	.28
Gentamicin (60)	—	0	0	0
*Piperacillin (600)	.76	.67	—	.33
Piperacillin (600)	.48	.55	—	.01
*Tetracycline (90)	1.12	1.16	—	.56
Tetracycline (90)	.20	.02	—	0
*Tobramycin (40)	—	1.05	1.06	.90
Tobramycin (40)	—	0	0	0

—Time point not included in reconstruction.

*Specimen transport system urine cocktail is present.

**Number in parenthesis represents final concentration (ug/ml) of antibiotic in urine

TNTC = too numerous to count

TABLE XVII-2

Antibiotic**	Hour Time Points			
	4	6	24	40
*No Drug	1.10	1.31	1.08	
No Drug	1.00	3.7-	TNTC	
		TNTC		
*Amikacin (210)	.86	.64	.27	
Amikacin (210)	0	0	0	
*Ampicillin (210)	1.13	1.18	.67	
Ampicillin (210)	1.30	TNTC	TNTC	
*Carbenicillin (710)	1.06	.83	.33	
Carbenicillin (710)	.15	.01	.03	
*Cefamandole (200)	1.15	1.07	.49	
Cefamandole (200)	.16	.11	.02	
*Cefobid (500)	.67	.90	.90	
Cefobid (500)	.007	.007	0	
*Cefotaxime (200)	1.00	1.33	3.83	
Cefotaxime (200)	.02	.008	.015	
*Cefoxitin (250)	.64	.80	.49	
Cefoxitin (250)	.04	.05	.14	
*Cephalothin (200)	.70	.81	.18	
Cephalothin (200)	.10	.02	.03	
*Chloramphenicol (180)	1.22	1.07	.59	
Chloramphenicol (180)	.84	.87	.39	
*Erythromycin (80)	1.27	1.00	1.08	
Erythromycin (80)	1.63	2.25	TNTC	
*Gantrisin (1000)	.82	.67	1.00	
Gantrisin (1000)	2.29	.60-	3.8-	
		TNTC	TNTC	
*Gentamicin (60)	.94	.73	.22	
Gentamicin (60)	0	0	0	
*Moxalactam (1000)	3.48	3.52	2.30	
Moxalactam (1000)	.11	0	0	
*Piperacillin (600)	.52	.84	.46	
Piperacillin (600)	1.07	.15	.34	
*Tetracycline (90)	.75	3.02	.23	
Tetracycline (90)	.90	.97	.23	
*Tobramycin (40)	.85	.95	.53	

TABLE XVII-2-continued

Antibiotic**	Klebsiella pneumoniae			
	4	6	24	
Tobramycin (40)	0	0	0	

—Time point not included in reconstruction.

*Specimen transport system urine cocktail is present.

**Number in parenthesis represents final concentration (ug/ml) of antibiotic in urine

TNTC = too numerous to count

TABLE XVII-3

Antibiotic**	Pseudomonas aeruginosa			
	4	6	24	
*No Drug	.92	.77	.53	
No Drug	1.48	2.49	TNTC	
*Amikacin (210)	1.33	1.41	.47	
Amikacin (210)	.58	.15	.01	
*Carbenicillin (710)	1.16	1.36	.80	
Carbenicillin (710)	.88	.38	.08	
*Moxalactam (1000)	1.10	.86	.41	
Moxalactam (1000)	.52	.18	.06	
*Piperacillin (600)	1.54	1.05	.91	
Piperacillin (600)	.32	.98	.23	
*Tobramycin (40)	.90	.42	0.5	
Tobramycin (40)	.22	.05	0	

—Time point not included in reconstruction.

*Specimen transport system urine cocktail is present.

**Number in parenthesis represents final concentration (ug/ml) of antibiotic in urine

TNTC = too numerous to count

TABLE XVII-4

Antibiotic**	Proteus vulgaris			
	4	6	24	
*No Drug	.57	1.07	3.11	
No Drug	.88	1.04	TNTC	
*Amikacin (210)	.72	.92	1.67-swarm	
Amikacin (210)	.15	0	0	
*Cefamandole (200)	.50	.50	.34	
Cefamandole (200)	.20	.21	.007	
*Piperacillin (600)	2.17	1.80	3.7-swarm	
Piperacillin (600)	0	0	0	
*Tobramycin (40)	.67	.92	swarm	
Tobramycin (40)	.18	.03	0	

—Time point not included in reconstruction.

*Specimen transport system urine cocktail is present.

**Number in parenthesis represents final concentration (ug/ml) of antibiotic in urine

TNTC = too numerous to count

TABLE XVII-5

Antibiotic**	Enterobacter cloaca			
	4	6	24	
*No Drug	1.0	.77	2.47	
No Drug	2.21	7.91	TNTC	
*Amikacin (210)	1.12	.62	.11	
Amikacin (210)	0	0	0	
*Ampicillin (210)	.99	1.68	3.60	
Ampicillin (210)	.18	.07	.02	
*Carbenicillin (710)	.76	.76	.14	
Carbenicillin (710)	.25	.23	.20	
*Cefamandole (200)	.89	1.44	1.11	
Cefamandole (200)	1.13	.48	.44	
Cefobid (500)	.07	.13	.04	
Cefobid (500)	.03	.003	.0005	
*Cefotaxime (200)	.92	1.04	.59	
Cefotaxime (200)	.12	.02	.05	
*Cefoxitin (250)	1.01	1.14	2.1	
Cefoxitin (250)	.25	.52	4.34	
*Cephalothin (200)	.88	1.03	.49	
Cephalothin (200)	2.41	4.37	TNTC	
*Chloramphenicol (180)	.97	.94	.94	
Chloramphenicol (180)	1.06	.97	.76	
*Erythromycin (80)	.95	1.04	1.06	

TABLE XVII-5-continued

Antibiotic**	Enterobacter cloaca			
	Hour	4	6	24
Erythromycin (80)		1.23	1.27	TNTC
*Gantrisin (1000)		.78	1.01	.99
Gantrisin (1000)		1.74	4.02	1.04-TNTC
*Gentamicin (60)		.68	.55	.13
Gentamicin (60)		0	0	0
*Moxalactam (1000)		5.28	5.68	5.80
Moxalactam (1000)		.10	0	0
*Piperacillin (600)		.91	.88	1.84
Piperacillin (600)		.65	.24	.09
*Tetracycline (90)		.85	1.73	1.23
Tetracycline (90)		1.03	2.43	.58
*Tobramycin (40)		.92	.81	2.12
Tobramycin (40)		0	0	0

—Time point not included in reconstruction.

*Specimen transport system urine cocktail is present.

**Number in parenthesis represents final concentration (ug/ml) of antibiotic in urine

TNTC = too numerous to count

TABLE XVII-6

Antibiotic**	Staphylococcus aureus				
	Hour	0	2	4	6
*SPECIMEN TRANSPORT SYSTEM URINE REGULAR URINE					
Antibiotic**		0	2	4	6
No Drug	1.00	—	.91	1.05	1.20
No Drug	1.00	—	.98	1.06	TN
*Amikacin (210)	1.00	—	.73	.37	.33
Amikacin (210)	1.00	—	.071	.008	.001
*Ampicillin (210)	1.00	.68	.70	—	.63
Ampicillin (210)	1.00	1.57	1.52	—	.86
*Carbenicillin (710)	1.00	.58	.57	—	.43
Carbenicillin (710)	1.00	.70	.80	—	.37
*Cefamandole (200)	1.00	.78	.68	—	.57
Cefamandole (200)	1.00	.70	.63	—	.14
*Cefobid (500)	1.00	—	1.15	1.15	1.17
Cefobid (500)	1.00	—	.58	.42	.08
*Cefotaxime (200)	1.00	—	.83	.82	1.00
Cefotaxime (200)	1.00	—	1.18	.82	.26
*Cefoxitin (250)	1.00	.79	.9	—	.76
Cefoxitin (250)	1.00	.79	.57	—	.21
*Cephalothin (200)	1.00	—	.87	1.2	2.03
Cephalothin (200)	1.00	—	.74	1.03	.39
*Chloramphenicol (180)	1.00	—	.73	.80	.63
Chloramphenicol (180)	1.00	—	.41	.28	.086
*Erythromycin (80)	1.00	—	.81	.85	.78
Erythromycin (80)	1.00	—	.23	.090	.012
*Gantrisin (1000)	1.00	—	.79	.84	.66
Gantrisin (1000)	1.00	—	.89	.21	2.22
*Gentamicin (60)	1.00	—	.71	.81	.36
Gentamicin (60)	1.00	—	0	0	0
*Moxalactam (1000)	1.00	—	.8	.76	.62
Moxalactam (1000)	1.00	—	.86	.65	.26
*Piperacillin (600)	1.00	.45	.58	—	.38
Piperacillin (600)	1.00	.72	.47	—	.19
*Tetracycline (90)	1.00	.64	.73	—	.73
Tetracycline (90)	1.00	.067	.012	—	0
*Tobramycin (40)	1.00	—	1.02	.99	.93
Tobramycin (40)	1.00	—	.093	.013	0

—Time point not included in reconstruction.

*Specimen transport system urine cocktail is present.

**Number in parenthesis represents final concentration (ug/ml) of antibiotic in urine

TNTC = too numerous to count

Tables XVII-1-XVII-6 clearly demonstrate the ability of the specimen transport system urine cocktail to block conventional therapeutic antimicrobials in the urine and to hold the microbial count relatively constant in the absence of antimicrobials.

It should be noted that with normal urine minus antibiotics the common pathogenic organisms will grow (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. vulgaris*, and *E. cloacae*) over a 24 hour period at room temperature. Hence, if the urine specimen is not analyzed promptly, it can lead to a false positive result. In the presence of

average urine concentrations of antibiotics (10x that of blood serum) sensitive pathogenic organisms rapidly die. This could lead the laboratory to the conclusion that the specimen does not contain a significant number of pathogenic organisms (10^5) when in reality the specimen did contain the pathogens at this level at the time of collection. In other words, if two or more hours have elapsed between collection and laboratory processing, the count obtained may be as low as 10^3 , i.e., considered not significant.

The urine specimen transport system achieves two major improvements, namely:

1. It is capable of effectively blocking the cidal effects of antibiotics for at least 6 hours, and in the most of cases, for up to 24 hours.

2. The number of organisms present at time zero in the presence or absence of antibiotics remains constant for up to at least 6 hours.

In conclusion, the unique features of this urine specimen transport system allows the urine specimen to be held for up to 24 hours prior to processing with no deleterious effect on the microbial integrity of the sample. Refrigeration is not required, and the system is effective in the absence or presence of antimicrobials.

As can be seen from the above examples, the specimen transport system which falls within the scope of the subject invention has many uses. The ability of the specimen transport system to hold the microbial count constant may allow for detection of significant microbial species which would otherwise be masked by the overgrowth of more rapidly dividing organisms.

EXAMPLE XVIII

Increasing Hypertonicity to Create Heightened Backtristostasis Effect

The following dry mixture was placed in each of a series of tubes:

0.03 grams of sodium polyanethosulfonate
0.005 grams of thioglycolate
0.1 grams of ICN free-base cysteine
0.1 grams of sodium bicarbonate

Various percentages, by weight thereof, of sodium chloride, indicated in Table XVIII-1 below were then added to individual tubes containing the above-described specimen transport system urine cocktail. A five milliliter aliquot of sterilized urine was then added to all tubes containing the above-described specimen transport system urine cocktail and to an equal number of tubes without the urine cocktail. Culture media containing *Enterobacter cloacae* ATCC #1344-2, was adjusted to a McFarlin of 0.5, representing approximately 5×10^8 microorganisms per milliliter of culture media, and then diluted 1:100 in sterile culture media. A 0.1 milliliter aliquot of diluted microorganisms was added to all urine containing tubes and the resulting mixture vigorously agitated. Ten microliter aliquots of the mixture were plated on agar plates such as described in Example VII. The remaining mixture was allowed to stand at room temperature for 24 hours after which time, a second ten microliter aliquot was plated as described in Example VII above. All plating was done in duplicate and the survival index (S-Factor) was calculated for each as described in the examples above. The average S-Factor for each time point was determined and is recorded in Table XVIII-1 below.

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TABLE XVIII-1

<i>Enterobacter cloacae</i> (ATCC # 1344-2)		S-Factor		
Sample	STS*	NaCl (%)**	0 Hour	24 Hours
1	—	—	1.00	TNTC***
2	+	—	1.00	1.72
3	+	1	1.00	1.27
4	+	2	1.00	.80
5	+	4	1.00	1.03
6	+	8	1.00	0.69

*STS Specimen transport system urine cocktail

**percentage percentage sodium chloride by weight

***TNTC too numerous to count

XVIII-2 below represents an average survival index (S-Factor) for quadruplicate plating.

The results, given in Table XVIII-2 below, confirm an increased stabilization of colony formation for all four strains of *Enterobacter cloacae* afforded by addition of about three percent, by weight, sodium chloride to the specimen transport system cocktail.

Hypertonicity may also be increased by utilizing other salts, carbohydrates or sugars. It is expected that appropriate concentrations to approach the effect of sodium chloride in this example may be calculated with the knowledge disclosed herein.

TABLE XVIII-2

<i>Enterobacter cloacae</i> (ATCC: various strains)		S-Factor HOUR TIME POINTS*										
Strain	—	4 + ADS	+ ADS + NaCl	—	6 + ADS	+ ADS + NaCl	—	24 + ADS	+ ADS + NaCl	—	48 + ADS	+ ADS + NaCl
1344-2	3.2	1.28	1.97	4.75-TN	1.23	.91	TN	3.88-TN	2.05	TN	TN	4.64-TN
3118	3.00	.87	.98	3.00-TN	.95	1.05	TN	4.25-TN	.98	TN	TN	4.14
2294	2.5-TN	1.00	.76	5.53-TN	.89	.89	TN	2.38-TN	.82	TN	TN	2.25
0879	2.5	.95	1.03	4.02-TN	.86	1.06	TN	TN	1.07	TN	TN	1.29-TN

*"—" indicates urine without specimen transport system cocktail; "+ ADS" indicates urine plus specimen transport cocktail; "+ ADS + NaCl" indicates specimen transport system cocktail containing 3 percent, by weight thereof, sodium chloride.

TN = too numerous to count.

In the absence of the specimen transport system urine cocktail, see Sample 1 in Table XVIII-1 above, the microorganisms present in the urine will quickly multiply and thus prevent the clinician from obtaining an accurate count of the number of microorganisms per milliliter of urine.

The results, displayed in Table XVIII-1 above, indicate that while the specimen transport system urine cocktail can decrease the rate of microbial replication, the addition of such salts as sodium chloride increase the effectiveness of the urine cocktail in holding the bacterial count, in urine, stable over a 24-hour period. The preferred range of salt, as determined from the results displayed in Table XVIII-1 above, is from about 2.5 percent to about 4.0 percent, by weight thereof. From the results of the salt titration experiment above, it was concluded that addition of about three (3) percent, by weight, sodium chloride to the specimen transport system urine cocktail should prevent the overgrowth of *Enterobacter cloacae* in urine over a 24-hour period. To verify this conclusion, the specimen transport system urine cocktail prepared as described above, was added to a series of tubes. A second series of tubes was prepared by adding the identical cocktail plus 0.15 grams of sodium chloride, the equivalent of three (3) percent, by weight, sodium chloride. A third series of tubes were set aside without cocktail. A five-milliliter aliquot of sterile urine was then added to each tube, including those tubes without cocktail. Four strains of *Enterobacter cloacae*, identified in Table 40 below, were grown separately and adjusted to a McFarlin of 0.5. Each strain was then diluted 1:100 in sterile culture media and a 0.1 milliliter aliquot of each added to individual urine tubes as identified in Table XVIII-2 below. After vigorous mixing, a ten-microliter aliquot from each tube was plated on agar plates as described in Example XVII. Thereafter, the mixture was allowed to stand at room temperature and at the time intervals indicated in Table XVIII-2 below, another ten (10) microliter aliquot was plated as described in Example VII. The results of each time point given in Table

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35 The following dry mixture was placed in a series of sterile tubes:

0.03 grams sodium polyanetholsulfonate
0.005 grams thioglycolate
0.1 gram of ICN free-base cysteine
0.1 gram of sodium bicarbonate
0.15 grams of sodium chloride

The various antibiotics, listed in Tables XIX-1-XIX-3 below, were added, at the concentrations also specified, to the tubes containing the above-described specimen transport system urine cocktail and to an equal number of tubes without the urine cocktail. Five milliliters of sterile urine was then added to all tubes after which the tubes were vigorously mixed. Control tubes contained either urine alone or urine plus the above-described specimen transport system urine cocktail. No antibiotics were added to control tubes. The microorganisms listed in Tables XIX-1-XIX-3 below were adjusted to a McFarlin of 0.5 and then diluted 1:100 with sterile culture media. A 0.1 milliliter aliquot of a single microorganism was added to each urine containing tube and the resultant mixture vigorously agitated. A ten-microliter aliquot from each tube containing the resultant mixture was immediately plated as described in Example XVII above. The tubes were then allowed to stand at room temperature for 24 hours. Additional ten microliter aliquots were plated as described in Example XVII above at the time points indicated in Tables XIX-1-XIX-3 below. All plating was done in quadruplicate, and the S-Factors recorded in Tables XIX-1-XIX-3 below represent an average of the quadruplicate plating.

The results set forth in Tables XIX-1-XIX-3 indicate that the salt containing specimen transport system urine cocktail was able to hold the colony count of *Proteus vulgaris*, *Streptococcus pneumoniae*, and *Streptococcus*

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pyogenes relatively stable in both the presence and absence of most antibiotics.

TABLE XIX-1

I. <i>Proteus vulgaris</i> (ATCC #23315)	S-Factor HOUR TIME POINTS*					
	4		6		24	
Antibiotic**	+	-	+	-	+	-
No Drug	1.06	1.07	1.38	3.15	1.12	TNTC
Ampicillin (210)	.77	.19	1.19	.04	.87	.004
Cefoxitin (250)	.73	.002	.79	.0008	.25	0
Chloramphenicol (180)	1.01	.53	.56	.32	.46	.06
Erythromycin (80)	.79	1.13	1.50	1.78	.94	1.07- TNTC
Gantrisin (1000)	1.47	.56	1.28	1.32	1.19	TNTC
Mezlocillin (500)	2.14	.31	1.51	.50	1.31	0

—Time point not included in reconstruction.

*Specimen transport system urine cocktail is present.

****Number in parenthesis represents final concentration of antibiotic in micrograms**

per milliliter of urine

TNTC = too numerous to count

TABLE XIX-2

I. <i>Streptococcus pneumoniae</i> (ATCC #23315)	S-Factor HOUR TIME POINTS*					
	4		6		24	
	+	-	+	-	+	-
No Drug	1.19	.82	.95	.73	1.27	4.80
Ampicillin (210)	1.60	.54	1.34	.43	1.08	.07
Cefamandole (200)	.42	1.04	.75	1.12	1.36	.28
Cefoxitin (250)	1.08	.98	1.11	.94	1.01	.20
Cephalothin (200)	1.04	.53	1.42	.53	2.54	.21
Chloramphenicol (180)	.57	.77	.80	.31	1.03	.30
Erythromycin (80)	.99	.73	.83	.73	.78	.32
Gantrisin (1000)	1.00	.96	1.00	.96	1.10	14.54
Mezlocillin (500)	1.18	.71	1.05	.29	1.03	.01

—Time point not included in reconstruction.

*Specimen transport system urine cocktail is present.

**Number in parenthesis represents final concentration of antibiotic in micrograms per milliliter of urine

TABLE XIX-3

1. <i>Streptococcus pyogenes</i> (ATCC #23315)	S-Factor HOUR TIME POINTS*					
	4		6		24	
Antibiotic**	+	-	+	-	+	-
No Drug	1.02	1.95	.93	3.10	.84	TNTC
Ampicillin (210)	.90	1.50	1.00	1.50	.79	1.00
Cefamandole (200)	.52	.41	.88	.09	1.28	.26

TABLE XIX-3-continued

1. <i>Streptococcus pyogenes</i> (ATCC #23151)	S-Factor HOUR TIME POINTS*					
	4		6		24	
Antibiotic**	+	-	+	-	+	-
Cefoxitin (250)	.60	.30	.94	.24	.96	.10
Cephalothin (200)	1.35	.63	1.50	.58	1.62	.08
Chloramphenicol (180)	.84	1.15	1.05	1.06	.75	.22
Erythromycin (80)	.54	.97	.33	.81	.54	.76
Gantrisin (1000)	.84	2.01	.94	2.34	.78	1.56
Mezlocillin (500)	.95	.74	1.33	.51	.62	.10
					TNTC	

—Time point not included in reconstruction.

*Specimen transport system urine cocktail is present.

****Number in parenthesis represents final concentration of antibiotic in micrograms per milliliter of urine**

TNTC = too numerous to count

EXAMPLE XX

EFFECTIVE CONCENTRATION OF SODIUM POLYANETHOLSULFONATE (SPS) FOR A SPECIMEN TRANSPORT SYSTEM TO PRESERVE MICROBIAL INTEGRITY AND ANTIBIOTIC-CONTAINING URINE SPECIMEN

The following dry mixture was placed in each of a series of sterile tubes:

0.005 grams thioglycolate
0.1 gram ICN free-base cysteine
01. gram sodium bicarbonate
0.15 grams sodium chloride

Different amounts of SPS, designated by weight percent thereof in Table XX-1 below, were added to the tubes containing the above-described specimen transport system urine cocktail and to the control tubes. The various antibiotics listed in Table XX-1 below were then added to one half of the tubes containing the above-described specimen transport system urine cocktail. Five milliliter aliquots of sterile urine were next added to all tubes. Control tubes containing urine but no antibiotic were established, half of which contained the above-described specimen transport system urine cocktail plus the various amounts of SPS designated in Table XX-1 below. *Staphylococcus aureus*, ATCC #25923, were grown, prepared and aliquoted into all tubes as described in Example XVII above. All tubes were plated as described in Example 11 above at the time points indicated in Table XX-1 below.

TABLE XX-1

<i>Staphylococcus aureus</i> (ATCC #25923)		S-Factor HOUR TIME POINTS*							
		2		4		6		24	
SPS %***	Antibiotic**	+	-	+	-	+	-	+	-
—	No Drug	ND	ND	ND	1.39	ND	1.50	ND	2.3-
0.6	No Drug	.70	ND	6.0	ND	ND	ND	.44	ND
1.0	No Drug	ND	ND	1.4	ND	.83	ND	.66	ND
2.0	No Drug	ND	ND	.85	ND	.83	ND	.91	ND
6.0	No Drug	.87	ND	.76	ND	ND	ND	.63	ND
0.6	Gentamicin (60)	.63	.02	.51	0	ND	ND	.51	0
1.0	Gentamicin (60)	ND	ND	1.12	0	1.03	.04	1.22	0
2.0	Gentamicin (60)	ND	ND	.75	0	.75	0	.47	0
6.0	Gentamicin (60)	.87	.12	.76	.01	ND	ND	.53	0
0.6	Tetracycline (90)	.64	.07	.73	.01	ND	ND	.6	0
1.0	Tetracycline (90)	ND	ND	1.0	0.1	1.3	0	.67	0
2.0	Tetracycline (90)	ND	ND	.68	0	.74	.007	.5	.002

TABLE XX-1-continued

<i>Staphylococcus aureus</i> (ATCC #25923)	SPS %***	Antibiotic**	S-Factor HOUR TIME POINTS*						
			2	4	6	24	+	-	
6.0	Tetracycline (90)		.91	1.09	.87	.34	ND	ND	ND

*Time point not included in reconstruction.

**Specimen transport system urine cocktail is present.

**Number in parenthesis represents final concentration of antibiotic in micrograms per milliliter of urine.

***Final sodium polyethoxylate concentration, by weight thereof, per tube.

TNTC = too numerous to count

+ Contains Specimen transport system.

- Does not contain Specimen transport system.

ND = Not Done

The results set forth in Table XX-1 above reveal an optimum range for SPS in the specimen transport system urine cocktail to be between about 0.6% and about 2.0%, by weight thereof.

While this invention has been described in relation to its preferred embodiments, it is to be understood that various modifications thereof will now be apparent to one skilled in the art upon reading the specification and it is intended to cover such modifications as fall within the scope of the appended claims.

I claim:

1. An apparatus for the collection and treatment of microorganisms in a specimen comprising:
 (a) a container of generally elongated shape having a first end and a second end;
 (b) a piston slidably mounted in said container;
 (c) a shaft attached to said piston and extending through said first end, said shaft includes a narrow portion causing said shaft to break at said narrow portion when said shaft is bent;
 (d) a detachable cap removably attached to said second end
 said cap, said container and said piston forming a sealed chamber, in which the pressure in the chamber may be decreased by moving said piston toward said first end;
 said cap being puncturable for insertion of said specimen, and;
 (e) a water-soluble specimen transport system deposited in said container, said specimen transport system comprising a water-soluble additive at a concentration effective to prevent replication of microorganisms present in said specimen, when said specimen is mixed with said water additive therein to form a solution, and reducing the cidal activity toward said microorganisms of antimicrobial factors present in said specimen so that at least some microorganisms will be capable of replication upon dilution of said solution on medium capable of supporting replication of said microorganisms.

2. The apparatus according to claim 1, wherein said apparatus further comprises a means to lock said piston to prevent movement of said piston within said container, said means to lock comprises the narrow portion of said shaft causing the shaft to break when said shaft is bent.

3. The apparatus of claim 1, further comprising:

(f) a substance effective for preserving the viability of microorganisms of interest in said specimen in said container.

4. The apparatus of claim 3, wherein said substance for preserving the viability of said microorganisms of interest comprises a growth base effective for support-

15 ing the general nutritional needs of the microorganisms of interest.

5. The apparatus according to claim 3, wherein said substance for preserving the viability of the microorganisms of interest in the specimen comprises starch.

6. The apparatus according to claim 3, wherein said substance for preserving the viability of the microorganisms of interest in the specimen comprises agar.

7. The apparatus according to claim 3, wherein said substance for preserving the viability of the microorganisms of interest in the specimen comprises hemoglobin.

8. An apparatus for the collection and treatment of microorganisms in a specimen comprising:

(a) a container of generally elongated shape having a first end and a second end;
 (b) a piston slidably mounted in said container;
 (c) a shaft attached to said piston and extending through said first end, said shaft being detachably connected to said piston by complementary thread means;
 (d) a detachable cap removably attached to said second end
 said cap, said container and said piston forming a sealed chamber, in which the pressure in the chamber may be decreased by moving said piston toward said first end;
 said cap being puncturable for insertion of said specimen, and;
 (e) a water-soluble specimen transport system deposited in said container, said specimen transport system comprising a water-soluble additive at a concentration effective to prevent replication of microorganisms present in said specimen, when said specimen is mixed with said water additive therein to form a solution, and reducing the cidal activity toward said microorganisms of antimicrobial factors present in said specimen so that at least some microorganisms will be capable of replication upon dilution of said solution on medium capable of supporting replication of said microorganisms.

9. The apparatus of claim 8 wherein said apparatus further comprises a means to lock said piston to prevent movement of said piston within said container, said means to lock comprises the complementary thread means allowing detachment of said shaft from said piston.

10. The apparatus of claim 8, further comprising:

(f) a substance effective for preserving the viability of microorganisms of interest in said specimen in said container.

11. The apparatus of claim 10, wherein said substance for preserving the viability of said microorganisms of interest comprises a growth base effective for support-

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ing the general nutritional needs of the microorganisms of interest.

12. The apparatus according to claim 10, wherein said substance for preserving the viability of the microorganisms of interest in the specimen comprises starch.

13. The apparatus according to claim 10, wherein said

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substance for preserving the viability of the microorganisms of interest in the specimen comprises agar.

14. The apparatus according to claim 10, wherein said substance for preserving the viability of the microorganisms of interest in the specimen comprises hemoglobin.

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United States Patent [19]

Newman et al.

[11]

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[45]

Sep. 16, 1980

[54] CULTURE COLLECTION AND TRANSPORT DEVICE

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[73] Assignee: Precision Dynamics Corporation, Burbank, Calif.

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[51] Int. Cl.² C12M 1/30

[52] U.S. Cl. 435/295; 128/759; 435/294; 435/810; 435/296; 435/300

[58] Field of Search 195/127, 139; 128/638, 128/743, 759; 435/292, 293, 294, 295, 810, 296, 299, 300

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[57] ABSTRACT

A culture collection and transport device which is uniquely configured employing a piston-like element which is adapted to maintain a medium-carrying chamber in fluid-tight relationship until such time as a specimen sample is introduced into the device and selectively put in contact with the medium. The culture medium preserves the viability of microorganisms comprising the specimen sample. The piston-like member is pressure responsive so that ambient temperature and pressure changes will not cause leakage of the medium from the medium-carrying chamber and wherein a simple, force oriented movement of a specimen collector on swab, containing a culture, is readily put into contact with the viability-maintaining, transport medium.

16 Claims, 9 Drawing Figures

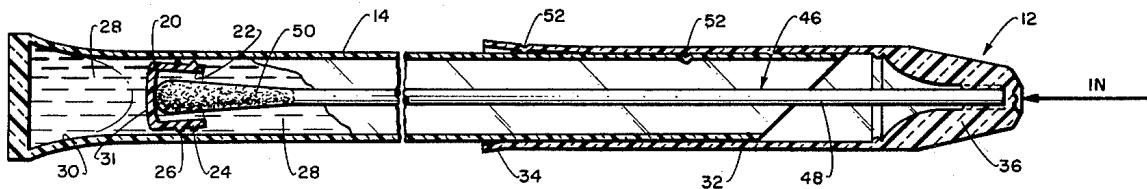


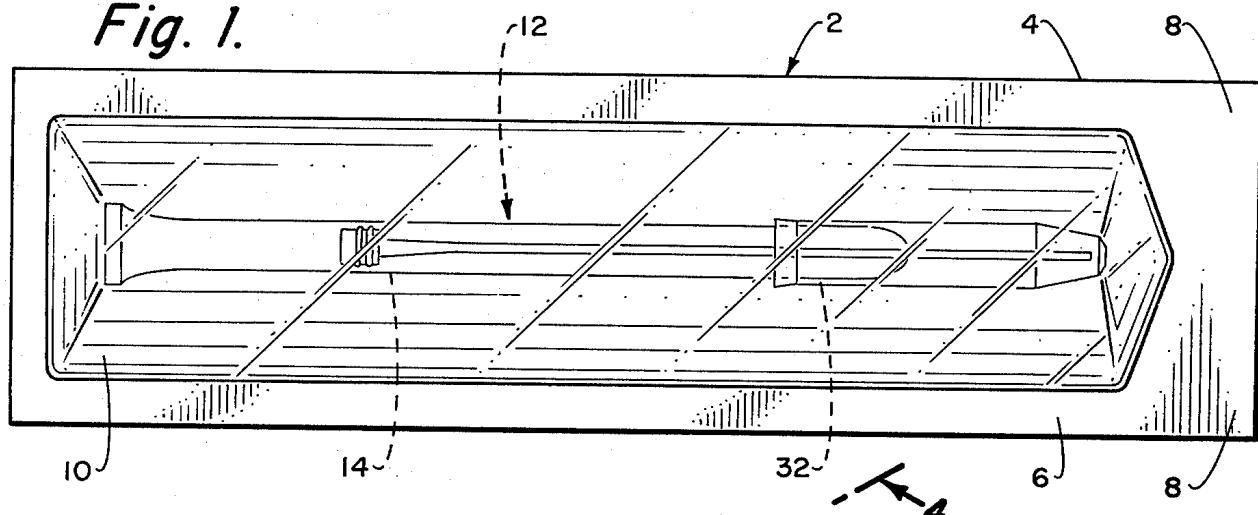
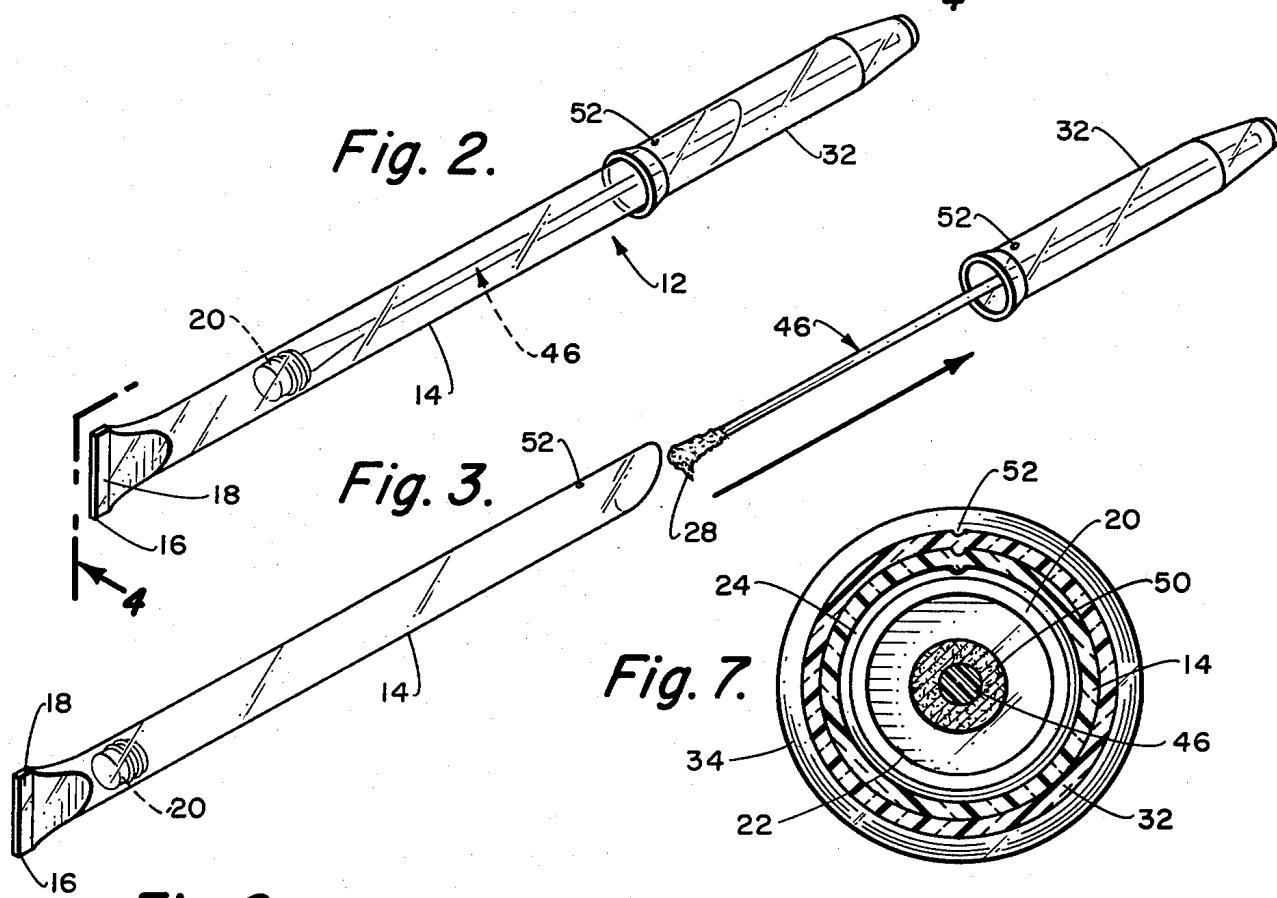
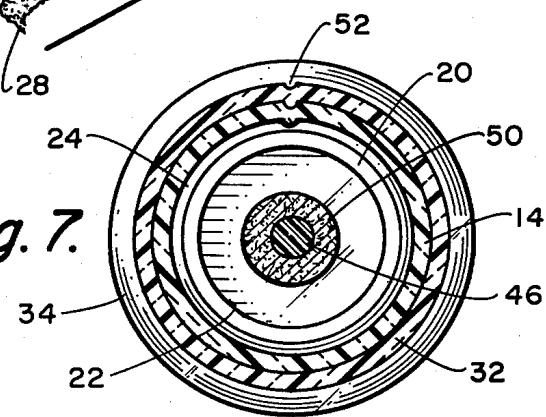
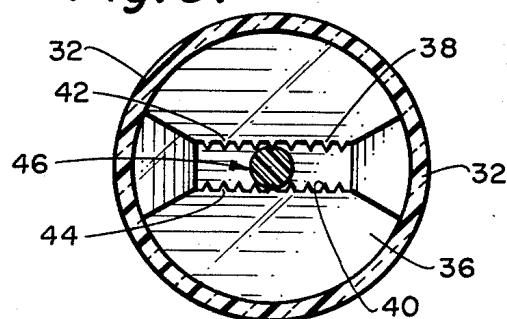
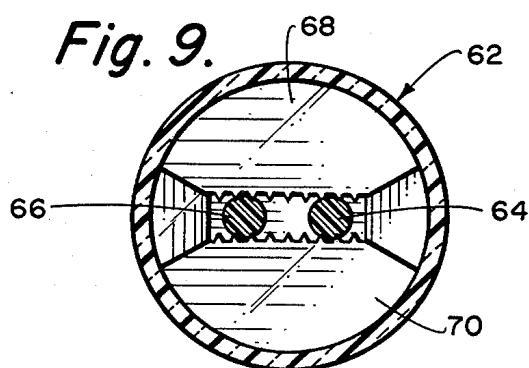
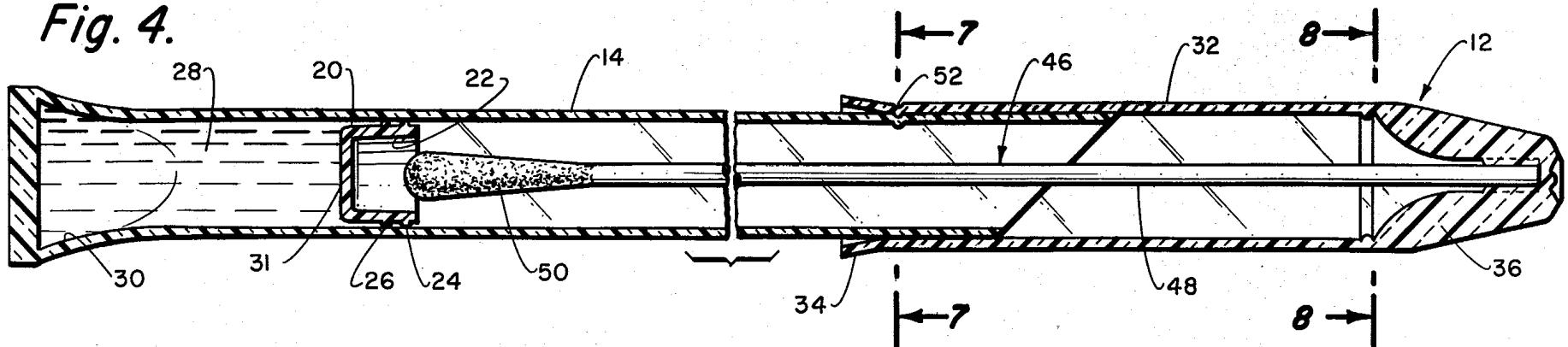
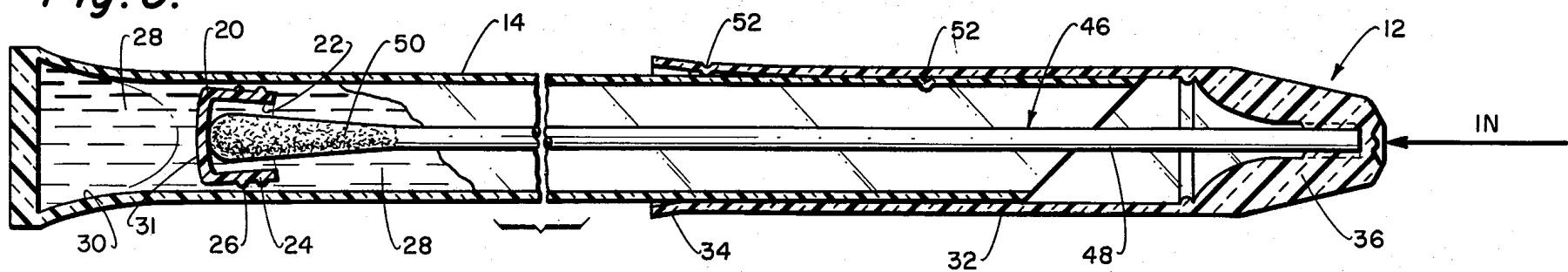
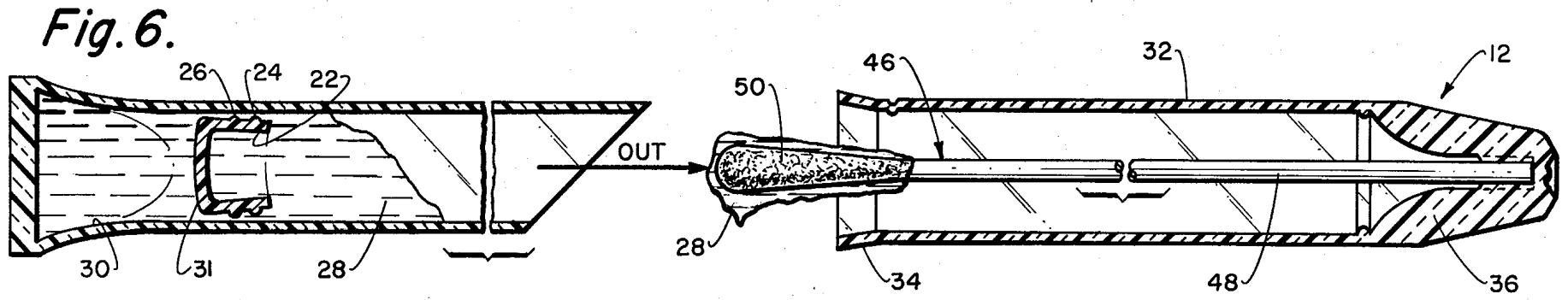
Fig. 1.*Fig. 2.**Fig. 3.**Fig. 7.**Fig. 8.**Fig. 9.*

Fig. 4.*Fig. 5.**Fig. 6.*

CULTURE COLLECTION AND TRANSPORT DEVICE

BACKGROUND OF THE INVENTION

This invention pertains to culture collection and transport devices of the type utilized in doctors' offices and the like wherein a specimen is obtained from a patient. In order to maintain the viability of the microorganism obtained as a specimen, until such time as the specimen can be tested by a testing laboratory, the microorganism must be maintained, and must be put in contact with a culture-sustaining medium, such as Amies, agar or other media that will preserve the viability of microorganisms for in vitro diagnostic test purposes.

In taking a culture specimen, a culture collection and transport device must be such that sterility of the specimen collector, e.g. swab, is maintained in a sterile environment and can be handled in an aseptic manner after the specimen of the microorganisms has been taken. Thereafter, the culture collection and transport device must be capable of furnishing a life-sustaining medium for the specimen microorganisms so that their viability will be maintained until such time as adequate laboratory tests may be made. Thus, a culture collection and transport device must be sterile before a test specimen is introduced, must have a microorganism-sustaining fluid or medium for the transportational phase and, subsequent to the specimen taking, must be capable of maintaining specimen integrity so as to give accurate test results. Additionally, because millions of cultures are taken annually, the devices must be economically and feasibly manufactured and must be of relative low cost because of their single-use character.

Prior art devices have utilized glass ampuls, partial and rupturable seals to maintain a culture-sustaining medium in isolation from the specimen collector, usually a swab or the like, until such time as the specimen of microorganisms has been obtained. The prior art devices suffer in that they are difficult to manufacture and to maintain quality control over, are costly in the manufacture of the various components making up the culture collection and transport device and generally suffer from other shortcomings, which the devices of this invention overcome.

OBJECTS AND SUMMARY OF THE INVENTION

It is an object of the invention to provide a culture collection and transport device which is suitable for in vitro diagnostic use.

It is another further object of the invention to provide a culture collection and transport device which employs a sealing member which is responsive to ambient pressure so as to prevent leakage of microorganism-maintaining medium.

It is still a further, and even more important, object of the invention to provide a culture collection and transport device which employs a piston-like member of unique configuration which maintains a culture medium chamber in fluid-tight relationship until such time as it is desired to disrupt that relationship.

It is still an even further, and more specific, object of the invention to provide a culture collection and transport device using an open-ended, tubular-like body member having a closed end which is adapted to carry a culture medium in one end thereof which is sealed off

from the remainder of the tubular-like body member by means of a piston-like member which is responsive to ambient pressure.

It is still another, even more specific and important, object of the invention to provide a culture collection and transport device which employs a piston-like member of conformable material wherein the piston-like member may provide a fluid-tight barrier between a body of culture medium and a specimen collector.

It is still another further, even more specific and important, object of the invention to provide a culture collection and transport device employing a cap-swab subassembly and a tube subassembly wherein the tube subassembly carries a culture medium maintained in a normally fluid-tight chamber by means of a piston-like, conformable member which is responsive to ambient pressure and which is selectively disengageable in order to provide culture medium contact with the specimen collector as desired.

It is still another, even more specific, and further object of the invention to provide a culture collection and transport device which employs two plastic tube members, one of which carries a culture medium separated from the remainder of the tube by a piston-like sealing member, and wherein the other tubular member carries a specimen collector such as a swab and wherein the two tubes fit together to form a single unitary aseptic culture taking and transport device.

It is another, even more further specific, object of the invention to provide a culture collection and transport device using a piston-like member which is of conformable material employing at least one perimetric sealing surface, protuberance or lip thereabout so as to provide a fluid-tight chamber for a culture medium carried in the device.

It is still a more further, and even more specific, object of the invention to provide a culture collection and transport device utilizing a piston-like member, employing perimetric sealing surfaces, protuberances or lips thereabout, which is of a unique configuration and design so as to be responsive to ambient pressure and to selectively permit collapse upon itself to disrupt the fluid-sealing character thereof.

In one embodiment, the invention pertains to a culture collection and transport device comprising the combination of an open-ended, tubular-like body member or tube subassembly having a closed end adapted to carry a fluid thereat and employing a conformable, piston-like member slideably positionable adjacent the closed end to form a normally, fluid-tight, chamber therebetween, in which a fluid medium is carried. The piston-like member has a least one perimetric, continuous, sealing surface, protuberance or lip thereabout in abutting relationship to the interior surface of said tubular-like body or tube subassembly member. The piston-like member is partially collapsible when subjected to selected applied pressure to open a fluid passage thereabout. A cap assembly member having disposed therein one or more specimen collectors and of a sufficient size to telescopically receive the open-ended, tubular-like body or tube subassembly member completes the major components of the culture collection and transport device.

These and further objects of the invention will become apparent from the hereinafter following commentary taken in conjunction with the figures of drawing.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is an elevational view showing the culture collection and transport device of the invention in an aseptic package;

FIG. 2 is a perspective view of the culture collection and transport device illustrated in FIG. 1, but being shown outside of the package in which it would normally be carried;

FIG. 3 is a view similar to FIG. 2 illustrating the major components making up the culture collection and transport device of the invention;

FIG. 4 is a view taken along the line 4—4 of FIG. 2;

FIG. 5 is a view similar to FIG. 4, but illustrating the mode of contacting the specimen collector with the culture medium carried by the culture collection and transport device of this invention;

FIG. 6 is a view similar to FIG. 5, but illustrating the culture medium and microorganisms retained on the tip of a specimen collector;

FIG. 7 is an enlarged view taken along the line 7—7 of FIG. 4;

FIG. 8 is an enlarged view taken along the line 8—8 of FIG. 4; and

FIG. 9 illustrates a section view of an alternative embodiment of the invention wherein the cap subassembly member carries more than one specimen collector or swab.

DESCRIPTION OF THE BEST EMBODIMENTS CONTEMPLATED

Referring to the drawings, wherein like numerals of reference designate like elements throughout, it will be seen that a package 2 contains a first paper sheet 4, heat or adhesively sealed to plastic over sheet 6 except at the corners 8 for ease of opening purposes, thereby forming a sterile chamber 10 in which is disposed the culture collection and transport device 12 of this invention.

The culture collection and transport device 12 comprises a first open-ended, tubular-like body member or tube subassembly 14 sealed at one extremity 16, leaving surface 18 to which may be affixed by heat stamp, imprinting or other means a lot number or identification to enable traceability throughout the life of culture collection and transport device 12. The open end of the member 14 is beveled for ease of association with a cap member as will be seen hereinafter. Also, ideally the tube diameter is sufficiently large to permit ease of access and egress of a specimen collector without wiping the specimen from the collector, as will be readily apparent.

Disposed in spaced relationship from the end 16 is piston-like member 20, which may be of natural or synthetic rubber or of one of the plastics, such as Krayton, a trademarked product of the Shell Company, or Polyurethane. The piston-like member 20 may be compression molded or injection molded and, depending upon the materials of construction, will be soft and conformable and have some lubricity, either by way of the materials of construction themselves or will utilize silicone oil or the like applied after fabrication or added to the materials making up the member 20 for purposes that will become apparent.

Piston-like member 20, in this particular instance, is of cup-like design having an interior recess 22 with spaced sealing lips or protuberances 24 and 26 about the perimeter of the outer wall of piston-like member 20. The piston-like member 20 is conformable and, generally,

will have a shore A rating in the 40-70 range for the size of piston-like member 20 utilized in the type of culture collection and transport device 12 illustrated in the figures of drawing. Thus, for general use purposes, a piston-like member 20 as illustrated will have a wall thickness of about 0.035 inches and a diameter of approximately 0.453 inches, it being understood that the length of the tube subassembly 14 being approximately 6.19 inches. It is only important that piston-like member 20 be capable of forming a fluid-tight barrier, as will be described, and be capable of being responsive to ambient pressures to which the culture collection and transport device 12 will be subjected. Further, as will be seen, the piston-like member 20 should be collapsible upon itself and be operative for its apparent purposes within an operating range of between 2-4 pounds or thereabout for the particular device being described, and it should be understood that those of ordinary skill in the art will understand the various modifications and changes that would necessarily be inherent should larger or smaller devices be desired, keeping in mind the foregoing parameters.

Positioned adjacent the closed end 16 of tubular-like body member or tube subassembly 14 is a quantity of culture medium 28 which may be Amies clear or charcoal, agar or any other type of generally suitable microorganism sustaining media well known in the art. Generally speaking, the fluid level of the culture medium 28 will be somewhat less than that amount that can be retained within the culture medium chamber 30 formed between the lower end 16 of tube subassembly member 14 and the bottom wall 31 of piston-like member 20 so that a quantity of air (not shown) will provide an air spring to cushion impact or shock loads on the medium chamber 30 due to ambient pressure, so that the piston-like member 20 may slide within the interior of tube subassembly 14 and still maintain the fluid-tight barrier between medium chamber 30 and the remainder of tube subassembly member 14.

Referring to FIG. 4, it will be seen that the piston-like member 20, by means of spaced sealing surfaces or lips 24 and 26, provides a fluid barrier to maintain substantial fluid integrity within medium chamber 30. The cap assembly member 32 is also of tubular configuration, having a slightly larger inside diameter so as to be telescopically received over the upper end portion of tube subassembly member 14. For ease of telescopic association, cap assembly member 32 has a flared end 34 and a closed end 36, molded in the interior surface to form opposed interior gripping surfaces 38 and 40 (FIG. 8) having spaced serrated projections 42 and 44 in which is frictionally retained a specimen collector or swab 46, the shaft 48 being made of plastic or the like with the tip 50 being of rayon absorptive material of the type generally found in the culture collection field.

Referring to FIG. 4, it will be noted that the overall length of specimen collector or swab 46 is such that room is left between the inside recess surface 22 (more particularly, the inside, bottom wall of piston-like member 20) and the outer periphery of rayon tip 50 of swab 46. Thus, as seen in FIG. 4, the piston-like member 42 may linearly move between the closed end 16 and the open end of tube subassembly member 14, due to the influence of ambient pressures acting upon the fluid medium 28 or air contained within fluid medium chamber 30. Because of the sealing protuberances or lips 24 and 26 and the coaction of the configuration of piston-like member 20 and the lubricity of the materials of

construction of both the piston-like member 20 and the tube subassembly 14, a fluid barrier or fluid tightness is achieved to prevent any contact of the fluid medium 28 with swab tip 50, except under desired and selected circumstances, as will be described.

In the FIG. 4 showing, the cap assembly member 32 is shown as being heat sealed or tack welded, as at 52, so as to join cap assembly member 32 to tube subassembly member 14 in releasable fashion. Thus, by simply twisting the cap assembly member 32 the tack weld or tamper indicator 52 seal will be broken to allow removal of the cap assembly 32 with its captively retained or staked swab 46, so that a microorganism specimen may be taken by means of swab tip 50.

Referring to FIG. 5, once the specimen has been collected, it is now imperative, for transportational purposes and to maintain the viability of the microorganism specimen, that the fluid medium be put into association with the tip 50. Thus, once the specimen is taken, the user of the culture collection and transport device would merely position the cap assembly member 32 with specimen collector 46, as illustrated in FIG. 2, and by means of using one hand and having the thumb in ballpoint pen actuating position, depress the outer end 36 of cap assembly member 32 in the direction of the arrow illustrated in FIG. 5 to thereby drive the tip 50, having the specimen thereon, into abutting engagement with the interior recess 22 of piston-like member 20, which because of the noncompressibility of the fluid medium 28 in fluid chamber 30 will cause the walls of piston-like member 20 to collapse about itself to thereby break the perimetric seals formed by sealing surfaces or members 24 and 26 to provide fluid paths for medium 28 to flow thereabout and contact the microorganisms contained on swab tip 50.

Referring to FIG. 6, obviously in order to remove the specimen and to make whatever microorganism test would be desirable, the cap assembly member 32 is removed, containing on the swab tip 50 not only the collected specimen in viable form, but a portion of the fluid medium 28 which has saturated the swab tip 50 in order to maintain and to preserve the viability of the collected specimen for in vitro testing purposes.

Referring now to FIG. 9, an alternative type of cap assembly 62 is illustrated identical in all particulars to cap assembly member 32 except, in this particular instance, two specimen collectors or swab members 64 and 66 are carried in frictionally retained relationship between the interior of sidewalls 68 and 70 making up the upper end of cap assembly 62. In all other particulars, the serrated interior surfaces of wall members 68 and 70 serve to frictionally retain the plastic shafts of swab members 64 and 66. Thus, with the dual concept, two specimen collectors are provided for specimen collection purposes.

In the specimen collection and transportational device 12 illustrated, it should be remembered that the specific description is for illustrative purposes only. For example, while the tube subassembly member 14 and the cap assembly member 32 are made of polyethylene, other materials of construction are, indeed, possible, keeping in mind the sliding parameter that is necessary for the piston-like member 20 with respect to the interior wall of the tube subassembly member 14. In some instances, the microorganism-sustaining fluid will be of varying colors and the piston-like member 20 may likewise be colored to accommodate the specific color of the medium with which it is to be used.

The piston-like member must have sufficient sealing integrity so as to be able to provide the fluid barrier between the culture media chamber and the remainder of the tube subassembly and, thus, the parameters of the material of construction of both the piston-like member, the tube subassembly and the relative sizes of each must be coordinated so that the forces that may cause bending of the swab shaft would be greater than the force necessary to break the fluid barrier maintained by the piston-like element, which, in turn, would be greater than the static friction force between the interior of the tube subassembly member and the conformable or elastomeric piston-like member.

The piston-like member and the interior wall of said tubular-like body member have relative coefficients of friction so as to permit said piston-like member to be responsive to fluid pressure changes in said normally fluid-tight chamber while maintaining fluid sealability about said piston-like member through said sealing lips. Thus, as indicated hereinbefore, the various component factors should provide an overall device that will only require between 2 and 4 pounds of force in order to break the fluid barrier formed by piston-like member 20 and still attain the aforealluded to advantages.

The culture collection and specimen device of the invention, of course, may be utilized without an outer package. An outer package like that illustrated is however, deemed necessary where exterior sterility of the culture collection and specimen device is desired. Additionally, in some instances, a cap assembly having the retained swab may be dispensed with and other alternative means utilized in order to obtain the specimen, in which event only the tube subassembly member need be utilized. Various changes and modifications will make themselves apparent to those of ordinary skill in the art, and all such changes will not depart from the essence of the invention as disclosed herein and as intended to be covered in the appended claims.

We claim:

1. A culture collection and transportational device comprising the combination of: an open-ended, tubular-like body member having a closed end adapted to carry a fluid therat; a conformable piston-like member slideably positionable adjacent said closed end to form a normally fluid-tight chamber therebetween in which said fluid medium is carried, said piston-like member having at least one perimetric continuous sealing lip thereabout in abutting relationship to the interior surface of said tubular-like body member, said piston-like member being partially collapsible when subjected to selected applied pressure to open a fluid passageway about the outer periphery of said piston-like member.

2. The device in accordance with claim 1 including a cap member adapted for telescopic association with said tubular-like body member.

3. The device in accordance with claim 2 wherein said cap member includes a specimen collector releasably carried thereby.

4. The device in accordance with claim 1 wherein said piston-like member is collapsible upon itself and has two spaced continuous sealing lips about the perimeter thereof, each of which is in abutting relationship to the interior surface of said tubular-like body member.

5. A device in accordance with claim 4 wherein a cap member is telescopically received on the open end of said tubular-like body member and one extremity thereof is adapted to receive the shaft of a specimen

collector in releasably, frictionally-held association therewith.

6. The device in accordance with claim 5 wherein said piston-like member is cup-shaped, wherein an open recess surface is projected to and positioned adjacent at the specimen end of said specimen collector. 5

7. The device in accordance with claim 6 wherein said piston-like member is made of conformable material having elastomeric characteristics.

8. The device in accordance with claim 7 wherein said piston-like member and the interior wall of said tubular-like body member have relative coefficients of friction so as to permit said piston-like member to be responsive to fluid pressure changes in said normally fluid-tight chamber while maintaining fluid sealability 15 about said piston-like member through said sealing lips.

9. The device in accordance with claim 8 including a quantity of culture medium disposed in said fluid-tight chamber and a gaseous space between the surface of said medium and the presented surface of said piston-like member, whereby an air cushion or spring is provided for absorption of relatively high impact forces so as to maintain fluid sealability of said piston-like member, and wherein fluid sealability is disrupted of said piston-like member by impacting the recessed end of 20 25 said piston-like member with said specimen collector.

10. The device in accordance with claim 6 wherein said cap member is adapted to receive two specimen collectors in friction held, side-by-side relationship and said cap member telescopically receives the exterior, 30 circumferential surface of said tubular-like body member.

11. The device in accordance with claim 6 wherein said tubular-like body member and said cap member are of heat-sealable plastic and wherein a tack weld is provided between said cap member and said tubular-like body member so as to provide a tamper evident seal. 35

12. A culture collection and transport device comprising the combination of: an open-ended plastic tubular body member having a sealed end; a conformable, 40 piston-like member slideably positioned from said sealed end and forming a fluid-tight chamber therebe-

tween, a quantity of fluid carried in said fluid-tight chamber, said piston-like member having spaced sealing surfaces coacting with the interior wall of said open-ended, plastic tubular body member to selectively maintain the fluid tightness of said fluid-tight chamber and being pressure responsive to disrupt said sealing surfaces upon the application of a selected fluid pressure within said fluid-tight chamber.

13. The culture collection and transport device in accordance with claim 12 including a plastic cap member carrying a swab and telescopically received over the upper end portion of the open end of said open-ended, plastic tubular body member to thereby cooperatively form an elongated, closed chamber.

14. The culture collection and transport device in accordance with claim 13 when said piston-like element is of relatively thin-wall, elastomeric-like material of construction, and has a recessed or cup-like surface adjacent the tip of said swab.

15. The culture collection and transport device in accordance with claim 14 wherein said spaced sealing surfaces are formed by spaced, continuous protuberances on the exterior surface of said piston-like member.

16. A culture collection and transport device comprising the combination of: an open-ended plastic tubular body member having a sealed end; a conformable, piston-like member slideably positioned from said sealed end and forming a fluid-tight chamber therebetween, a quantity of fluid carried in said fluid-tight chamber, said piston-like member having spaced sealing surfaces coacting with the interior wall of said open-ended, plastic tubular body member to selectively maintain the fluid tightness of said fluid-tight chamber and being pressure responsive to disrupt said sealing surfaces upon the application of a selected fluid pressure within said fluid-tight chamber, and a plastic member carrying a swab and being telescopically received over the upper end portion of the open end of said open-ended, plastic tubular body member to thereby cooperatively form an elongated, closed chamber.

* * * * *

April 14, 1970

C. M. HUCK

3,506,008

MEDICAL APPLICATOR

Filed March 25, 1968

Fig. 1.

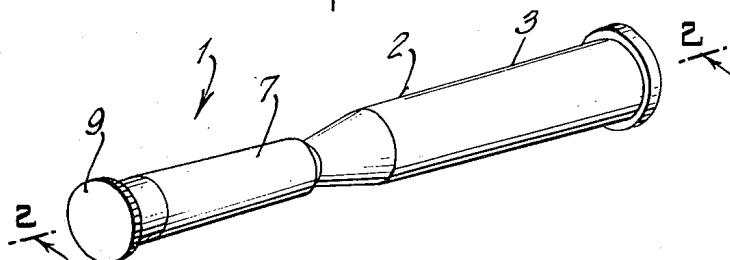


Fig. 2.

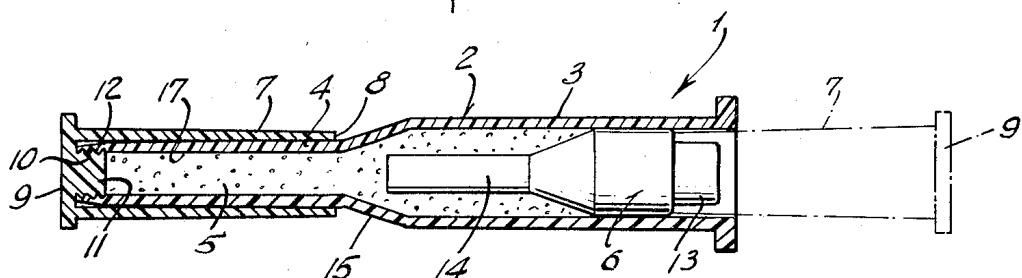


Fig. 3.

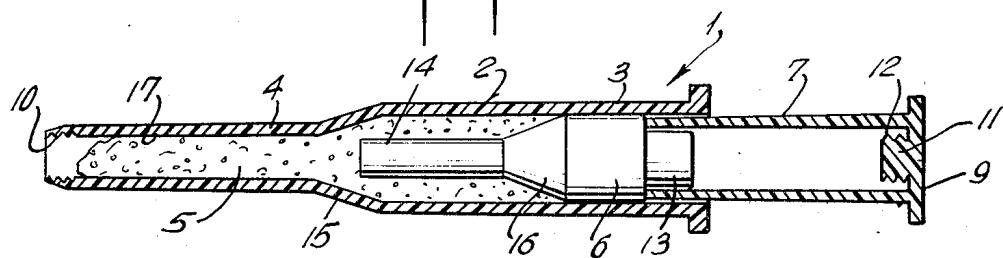
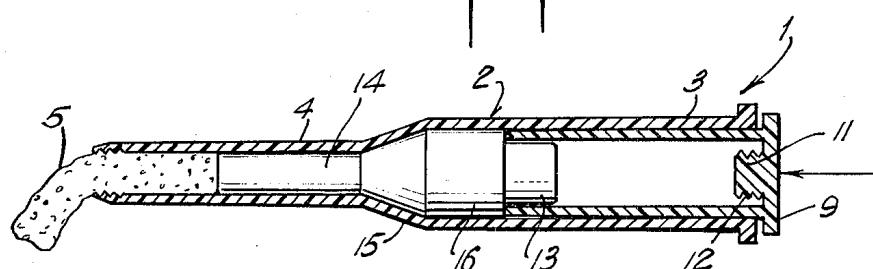


Fig. 4.



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3,506,008
MEDICAL APPLICATOR
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Int. Cl. A61m 35/00
U.S. CL. 128—261

5 Claims

ABSTRACT OF THE DISCLOSURE

A prefilled syringe type applicator wherein the plunger is hollow and is initially telescopically and disengageably disposed over the ejector tip thus serving to seal and protect the same. A plug capable of doubling as a leader for the plunger is slidably mounted at the open end of the medicament reservoir. The plunger when disengaged from the ejector tip is engageable with the outward facing end of the plug to thus provide an applicator ready for use.

BACKGROUND OF THE INVENTION

Prior art

For many medical reasons it is necessary or desirable to apply various medicaments to body cavities. In order to provide a simple method of applying the medicament with a minimum of unpleasantness, the medicaments are often applied with a syringe type applicator. Most often the patient purchases the syringe prefilled with the medicament which has been prescribed at the desired dosage level.

Conventional applicators of this type have a plastic body portion, one end of which constitutes a medicament reservoir and the other end of which is tapered to relatively small diameter and constitutes an ejector tip. A plunger, the leading end of which is of only slightly smaller diameter than the medicament reservoir, is telescopically mounted within the reservoir, thus sealing one end of the applicator. The end of the ejector tip is breakably sealed. When the time has come to apply the medicament, the user breaks the seal at the end of the ejector tip, inserts the ejector tip into the body cavity to which the medicament is to be applied, and presses the plunger to force the medicament out the open end of the ejector tip.

Deficiencies of the prior art

The above described syringe-type applicators have a number of inherent deficiencies. First, such a syringe is not easily adaptable to more than one use since once the ejector tip seal is broken and a portion of the medicament applied, no means is provided to reseal the partially emptied syringe for storage until the medicament is again needed. This may result in a waste of expensive medicament, and, if makeshift sealing is attempted, may result in inadvertent ejection of the medicament during storage. Too much pressure on the plunger during transportation or storage prior to initial use may cause the ejector tip seal to break prematurely, thus resulting in unsalable goods, in the case of the distributor and retailer, or wasted medicament and a messy condition in the case of the user.

Secondly, these conventional syringe-type applicators consisting of the plunger, medicament reservoir, and ejector tip consecutively arranged, are of substantial length and thus not adaptable to efficient and compact packaging or storage. These applicators are also not readily adaptable to being carried in a discrete manner by the patient for use outside of the home.

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Thirdly, a major portion of the ejector tip is exposed during transportation and storage and between uses, thus raising the possibility of gross contamination which may be introduced into the patient.

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THE INVENTION (IN GENERAL)

Now, in accordance with this invention, there is provided a prefilled syringe-type applicator of medicament which eliminates to a large degree the above-noted deficiencies of presently available syringe-type applicators.

The applicator of this invention comprises an elongated body portion open at both ends and having an integrally jointed first section and second section the first section being of relatively uniform cross section and constituting a medicament reservoir which is, in fact, filled with medicament, and the second section constituting an ejector tip. The applicator also comprises a plug which at its point of largest cross section dimension is of the same shape as and only slightly smaller than the inside of the medicament reservoir. This plug is slidably disposed within the medicament reservoir at its open end thus acting to seal the same. The applicator further comprises a hollow plunger the first end of which is open, the inner dimensions of which are of larger size than the outer dimensions of the ejector tip and the outer dimensions of which are of smaller size than the inner dimensions of the medicament reservoir so that the plunger can be and is telescopically mounted over the ejector tip. The inner surface of the marginal end portion of the ejector tip carries a series of fastening elements. The second end of the plunger has a centrally located inward projection of similar shape as and of only slightly smaller size than the inner dimensions of the marginal end portion of the ejector tip. This projection carries a series of fastening elements which are complementary with and in fact engaged with the fastening elements on the inner surface of the marginal end portion of the ejector tip; the projection acting as a plug for the ejector tip. The outward facing end of the plug and the open end of the plunger also have complementary fastening elements so that the plunger may be secured to the end of the plug.

By virtue of the construction in which the plunger is initially telescopically mounted over the ejector tip, a syringe-type medicament applicator is provided which is more compact and thus more readily stored and discreetly carried than prior art applicators.

By virtue of the projection within the plunger acting as a plug for the ejector tip and by virtue of the complementary fastening means carried by the projection and the inner surface of the end of the ejector tip, any messy or inconvenient seal breaking prior to application of the medicament is eliminated. When the medicament is to be used the plunger is simply unfastened from the ejector tip to open the same and to allow the medicament to flow from the applicator when the plug is activated. By virtue of these same features, the applicator of this invention may be used for a plurality of measured doses since the end of the ejector tip may be sealed again mounting the plunger over the tip and engaging the fastening elements.

By virtue of the plunger being mounted over the ejector tip, the tip, which is to be inserted into the body cavity, is protected from gross contamination by bacteria during storage. In addition, since the ejector tip fastening elements are located on the inner surface of the same, the outer surface remains smooth and thus non-abrasive. The invention will be better understood by reference to the attached drawings and the description of the same which follows:

In the drawings

FIGURE 1 is a view in perspective of one prototype of this invention;

FIGURE 2 is a cross section of the applicator illustrated in FIGURE 1 taken along lines 2—2;

FIGURE 3 illustrates in cross section the applicator of FIGURES 1 and 2 when the same is ready for use;

FIGURE 4 illustrates in cross section this same applicator in use.

Referring now to FIGURES 1 and 2, the applicator 1 comprises an elongated body portion 2 of circular cross section and open at both ends. The elongated body portion 2 in turn consists of first section 3 and second section 4 integrally joined by central section 15. The first section 3 constitutes a medicament reservoir which is in fact filled with medicament 5. The second section of the body portion 4 constitutes the ejector tip through which the medicament is applied. In general, the ejector tip 4 will, as illustrated, have a substantially smaller diameter than the medicament reservoir 3. As illustrated, the body portion of the applicator is made of a molded plastic by techniques well-known in the art, however, any suitable material may be utilized. Likewise, the elongated body portion is of circular cross section, however, as is apparent, the shape of the body portion is not critical to the invention.

The applicator 1 also comprises a rubber plug 6 having a maximum diameter which is slightly smaller than the inner diameter of the medicament reservoir 3. The rubber plug 6 is slidably disposed in the open end of the medicament reservoir 3 thus acting to seal the same. While as illustrated, the plug 6 is made of rubber, it may also be made of inert plastic or any other suitable, relatively inert, nontoxic material.

The applicator 1 further comprises a hollow molded plastic plunger 7 having open end 8 and closed end 9. The plunger 7 has an inner diameter greater than the outer diameter of the ejector tip 4 and an outer diameter less than the inner diameter of the medicament reservoir 3. In the preferred embodiment illustrated the compactness of the applicator is maximized by making the inner diameter of the plunger 7 only slightly greater than the outer diameter of the ejector tip 4 and the outer diameter of the plunger 7 only slightly less than the inner diameter of the medicament reservoir 3. The plunger 7 is telescopically mounted over the ejector tip 4, the ejector tip and the plunger being of substantially the same length.

The inner surface 17 of the marginal end portion of the ejector tip 7 contains a series of threads 10 integrally molded therein. The closed end 9 of the plunger contains an inward projection 11 having a diameter only slightly less than the inner diameter of the marginal end portion of the ejector tip and having a series of threads 12 integrally molded in the outer surface of the same. These threads 12, carried by the projection 11, are engaged with the threads 10 carried by the ejector tip 4. Thus, the projection 11 acts as a plug for the ejector tip 4 thus preventing medicament from passing outward from the same. While as illustrated the fastening means on the outer surface of projection 11 and the inner surface 17 of the ejector tip 4 are intermeshing threads, any complementary fastening means which may be easily engaged and disengaged may be utilized.

The outward facing end of the plug 6 has a portion 13 having a diameter only slightly smaller than the inner diameter of the plunger 7. Thus, the plunger 7 when it is disengaged from its telescopic mounting over the ejector tip 4 may be inserted over and engaged with the end 13 of the plug 6 as shown in FIGURE 3. While as illustrated in FIGURE 3 the connection between the plug and the plunger is merely by virtue of relative shapes and sizes of the two members, any conventional and suitable fastening means may be provided.

Referring now to FIGURES 3 and 4, when it is desired to apply the medicament, the plunger 7 is unscrewed from its position with respect to ejector tip 4 and is inserted over the end 13 of the plug 6, so that the plunger is in effective position to activate the plug 6 and force the medicament 5 in the case illustrated, a vaginal cream, outward from the ejector tip 4.

In order to most efficiently dispense the medicament it is preferable that a portion of the plug 6 have a shape as illustrated which is essentially the same as that of the inner surface of the central section 15 and adjacent portions 14 and 16 of the ejector tip 4 and medicament reservoir 3. Thus, when the plunger is fully depressed as shown in FIGURE 4, a maximum amount of the medicament will have been ejected from the applicator.

While particular embodiments of the invention have been illustrated and described, it is not intended to restrict the invention to those specific embodiments. Rather, the broad inventive concept as set out earlier is to be restricted only by the following claims.

What is claimed is:

1. An applicator comprising a hollow elongated body portion open at both ends and having an integrally joined first section and second section,

the first section being of relatively uniform cross section and constituting a medicament reservoir which is in fact filled with medicament, the second section constituting an ejector tip,

the applicator also comprising a plug which at its point of largest cross sectional dimension is of the same shape as and only slightly smaller than the inside of the medicament reservoir, the plug being slidably disposed within the medicament reservoir at its open end thus acting to seal the same,

the applicator still further comprising a hollow plunger, the first end of which is open, the inner dimensions of which are of larger size than the outer dimensions of the ejector tip and the outer dimensions of which are of smaller size than the inner dimensions of the medicament reservoir so that the plunger can be and is telescopically mounted over the ejector tip,

the inner surface of the marginal end portion of the ejector tip carrying a series of fastening elements and the second end of the plunger having a centrally located inward projection of similar shape as and of only slightly smaller size than the inner dimensions of the marginal end portion of the ejector tip, the projection carrying a series of fastening elements which are complementary with and in fact engaged with the fastening elements on the inner surface of the marginal end portion of the ejector tip, the projection acting as a plug for the ejector tip,

the outward facing end of the plug and the open end of the plunger also having complementary fastening elements so that the plunger may be secured to the end of the plug when the medicament is to be applied.

2. The applicator of claim 1 in which said elongated body portion and said plunger are generally of circular cross section and in which the plug at least its point of largest cross sectional dimension is of circular cross section,

said ejector tip having a diameter less than the diameter of the medicament reservoir, the inner diameter of the plunger being only slightly greater than the outer diameter of the ejector tip, and the outer diameter of the plunger being only slightly less than the inner diameter of the medicament reservoir.

3. The applicator of claim 2 in which the fastening elements on the inner surface of the marginal end portion of the ejector tip and the fastening elements on the outer surface of the inward projection of the plunger being intermeshing threads integrally molded into the respective components.

4. The applicator of claim 3 in which the outward facing end of the plug has a portion having a diameter only slightly smaller than the inner diameter of the plunger so that the plunger may be engaged with the plug by positioning the open end of the plunger over the plug.

5. The applicator of claim 4 in which the elongated body portion additionally comprises a central section integrally connecting said first section with said second section, and in which the plug has a shape which is essentially the same as that of the inner surface of the central section of the elongated body portion and the immediately adjacent portions of the ejector tip and the medicament reservoir.

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(54) 【発明の名称】細胞保存容器

(57) 【特許請求の範囲】

【請求項1】

細胞含有液を内部に収納可能な細胞収容部を備えた外筒と、
前記外筒内部に摺動可能に挿入され、細胞収容部の容積を可変可能なように押圧力の加減
が可能なピストンを備えた

注射器仕様の細胞保存容器であって、

前記細胞収容部の内面が、ポリアクryル酸、ポリメタクリル酸からなる群より選ばれた材
料が結合または被覆された材料で形成され、

前記細胞保存容器の先端には、細胞保存容器内部に対して外部と連通可能な注射針接続部
が設けられてなり、

前記注射針接続部の開口により、細胞含有液を内外に移送可能な
ことを特徴とする細胞保存容器。

【請求項2】

前記細胞収容部が、可撓性材料から形成され、前記ピストンの押圧力の加減により変形可
能な袋状容器からなり、前記袋状容器が前記外筒内部に収容されてなる

ことを特徴とする請求項1に記載の細胞保存容器。

【請求項3】

前記袋状容器がチューブ状に形成されてなり、押圧力の加減により、細胞含有液の内外の
移送が可能な

ことを特徴とする請求項2に記載の細胞保存容器。

【請求項4】

前記袋状容器が蛇腹状に形成されてなり、押圧力の加減により、細胞含有液の内外の移送が可能な

ことを特徴とする請求項2に記載の細胞保存容器。

【請求項5】

前記注射針接続部が、前記袋状容器の先端に設けられてなり、前記外筒先端部の開口部より突出するよう構成された

ことを特徴とする請求項2から4の何れかの項に記載の細胞保存容器。

【請求項6】

前記細胞保存容器の外筒に袋破壊手段が設けられた

ことを特徴とする請求項2から4の何れかの項に記載の細胞保存容器。

【請求項7】

前記細胞収容部の一部または全部が、ガス透過性材料で形成されてなる請求項1～6の何れかの項に記載の細胞保存容器。

【請求項8】

ガス透過性材料が、ガス透過性の良好な合成樹脂で形成された非多孔質のシートである請求項7記載の細胞保存容器。

【請求項9】

ガス透過性の良好な合成樹脂が、シリコーン樹脂、ポリ4メチルペンテン1、ポリイソブレン、ポリブタジエン、エチレン酢酸ビニル共重合体、低密度ポリエチレンまたはポリスチレンである請求項8記載の細胞保存容器。

【請求項10】

ガス透過性材料が、疎水性材料からなる多孔質膜である請求項8記載の細胞保存容器。

【請求項11】

容器側壁の少なくとも一部が多孔質膜で形成されてなる請求項10記載の細胞保存容器。

【請求項12】

多孔質膜が注射針接続部に装着されたキャップに取り付けられてなる請求項10記載の細胞保存容器。

【請求項13】

多孔質膜の孔径が1μm以下である請求項10～12の何れかの項に記載の細胞保存容器。

【請求項14】

多孔質膜の孔径が0.4μm以下である請求項10～12の何れかの項に記載の細胞保存容器。

【請求項15】

疎水性材料が、ポリテトラフルオロエチレン、テトラフルオロエチレン-ヘキサフルオロプロピレン共重合体、ポリエチレンテレフタレートまたはポリプロピレンである請求項10～14の何れかの項に記載の細胞保存容器。

【請求項16】

請求項1～15の何れかの項に記載の容器に細胞含有液が充填されてなる細胞製品。

【請求項17】

細胞製品が、治療用である請求項16記載の細胞製品。

【請求項18】

細胞が胚性幹細胞または体性幹細胞である請求項16または17記載の細胞製品。

【請求項19】

細胞が骨細胞である請求項16または17記載の細胞製品。

【請求項20】

細胞が軟骨細胞である請求項16または17記載の細胞製品。

【請求項21】

細胞が筋細胞である請求項16または17記載の細胞製品。

【請求項22】

細胞が心筋細胞である請求項16または17記載の細胞製品。

【請求項23】

細胞が神経細胞である請求項16または17記載の細胞製品。

【請求項24】

細胞が腱細胞である請求項16または17記載の細胞製品。

【請求項25】

細胞が脂肪細胞である請求項16または17記載の細胞製品。

【請求項26】

細胞が臍細胞である請求項16または17記載の細胞製品。

【請求項27】

細胞が肝細胞である請求項16または17記載の細胞製品。

【請求項28】

細胞が皮膚細胞である請求項16または17記載の細胞製品。

【請求項29】

細胞が血球系細胞である請求項16または17記載の細胞製品。

【発明の詳細な説明】**【0001】****【発明の属する技術分野】**

本発明は、内部に細胞を保存することができ、細胞が必要になった時には、面倒な操作をすることなく、ただちに容器から細胞を取り出して使用することができる細胞保存容器に関する。さらに詳しくは、再生医療などにおいて治療に細胞を使用する際に、そのまますぐに生体に注入できるように工夫した細胞保存容器に関する。

【0002】**【従来の技術】**

近年、細胞生物学や細胞工学の進歩により、生体から取り出した多分化能と自己複製能を持つ細胞（幹細胞）を患者に移植して治療を行う再生医療の研究が急速に進められている。これは標的細胞へ分化誘導をかけた幹細胞を生体組織の欠損部や疾患の責任病巣部位へ移植して目的組織（臓器）を修復・回復させる治療法である。現在はまだ基礎的研究の段階であるが、臨床試験も一部で試みられ始めている。このような場合、一般には、細胞を単独または足場の存在下に専用の容器内で培養した未分化な細胞または分化した細胞を、外科手術によって目的部位に移植する。

【0003】**【発明が解決しようとする課題】**

上述したような細胞の移植方法では外科手術が必要となるため、患者への負担が大きい。そこで、細胞を直接生体内に注入する方法が検討されている。とくに関節への軟骨細胞またはその前駆細胞の注入、脳への神経細胞またはその前駆細胞の注入、心臓への心筋細胞またはその前駆細胞の注入などは、有力な治療法となり得る。このような治療を実施する場合、従来の方法では、増殖して容器壁に接着している細胞をトリプシンやEDTAなどで処理して剥離し、洗浄などの工程を経て所定量を注射器に採取し生体に注入することになる。

【0004】

しかし、このような方法では操作の工程が多く面倒であるだけでなく、一連の操作は周囲からの汚染が起こらないようにクリーンな環境下で熟練した者が行う必要がある。このため、設備の整った施設以外では細胞移植治療を実施するのが難しい。

【0005】

本発明の目的は、細胞移植治療を簡便に実施することができる医療器具を提供することにある。すなわち、細胞の保存及び生体への注入を誰でも容易に行うことができる医療器具を提供するものである。

【0006】

【課題を解決する手段】

本発明においては、細胞含有液を内部に収納可能な細胞収容部を備えた外筒と、前記外筒内部に摺動可能に挿入され、細胞収容部の容積を可変可能なように押圧力の加減が可能なピストンを備えた注射器仕様の細胞保存容器の構成とし、前記細胞収容部の内面をポリアクリル酸、ポリメタクリル酸からなる群より選ばれた材料が結合または被覆された材料で形成した容器に増殖させた細胞を保存することにより、上述した課題を解決した。すなわち本発明は、細胞収容部の内面が、ポリアクリル酸、ポリメタクリル酸等の細胞の接着しにくい材料で形成されてなる細胞保存容器である。容器内面を細胞の接着しにくい材料で形成することにより、保存中に細胞が容器壁面に接着することがなくなり、細胞を容器壁面から剥離する操作が不用になる。なお、「細胞が接着しにくい」とは、細胞保存中に細胞が全く接着しないかあるいは接着しても簡単に剥がれる程度にしか接着しないことを意味する。また、本発明は注射器仕様の細胞保存容器であり、比較的簡単な操作により、細胞含有液の細胞保存容器の内外への移送が容易に可能になる。

【0007】**【発明の実施の形態】**

本発明の細胞保存容器は、内面が細胞の接着しにくい材料で形成されているが、容器を注射器の機能を有するものにしておけば、そのまま生体に注入することができるので便利である。また、容器の一部または全部をガス透過性の材料で形成すれば、密封状態でも細胞の生存に必要な酸素や炭酸ガスが透過できるようになり、細胞の長期保存が可能になるので好ましい。

【0008】**【実施例】**

本発明において使用する細胞の接着しにくい材料としては、表面が親水性材料または疎水性材料で形成されたもの及び表面に負電荷を有する材料をあげることができる。親水性材料としては対水接触角が50度以下のものが好ましく、疎水性材料としては対水接触角が100度以上のものが好ましい。好ましい親水性材料の例としては、アクリルアミド系重合体、メタクリルアミド系重合体、ポリアクリル酸、ポリメタクリル酸、ポリビニルアルコール、ポリエチレングリコール、ポリビニルピロリドン、セルロース、デキストラン、ヒアルロン酸、グリコサミングリカン、プロテオグリカン、カラギーナン及びタンパク質などを基材の表面にグラフト共重合や化学反応などの方法で結合するか表面に被覆した材料をあげることができる。また、疎水性材料としては、ポリテトラフルオロエチレン、テトラフルオロエチレン-ヘキサフルオロプロピレン共重合体などのフッ素樹脂及びシリコーン樹脂をあげることができる。また、表面に負電荷を有する材料としては、ポリアクリル酸、ポリメタクリル酸、スチレンスルホン酸、アルギン酸、ヘパリン、ヘパラン硫酸、コンドロイチン硫酸またはデルマタンを表面に結合した材料をあげることができる。これらの中でも、表面にカルボキシル基を有する材料がとくに好ましい。特に好ましい材料としては、ポリアクリル酸、ポリメタクリル酸が挙げられる。その理由は、上述した親水性材料としての細胞非接着効果と、負電荷による細胞非接着効果の両方の効果を有する材料であるからである。また、材料の表面は平滑な方が細胞の非接着性に優れているので好ましい。

【0009】

本発明において、容器の一部または全部をガス透過性材料で形成する方法としては、容器の側壁の一部または全部をガス透過性の良好な非多孔質材料で形成する方法と、容器の一部に多孔質膜を装着してガス交換が行われるようにする方法をあげることができる。

【0010】

ガス透過性の良好な材料としては、シリコーン樹脂、ポリ4メチルペンテン1、ポリイソブレン、ポリブタジエン、エチレン酢酸ビニル共重合体、低密度ポリエチレン及びポリスチレンなどをあげることができる。これらの材料は、プラスチック材料の中では比較的良好なガス透過性を有しているが、厚さが厚くなるとガス透過性が低下するので、通常は200μm以下であることが好ましく、100μm以下がとくに好ましい。本発明において

は、容器全体をこのような材料で形成することもできるし、一部だけをそのような材料で形成してもよい。必要なガス透過度は、容器の表面積、細胞の充填量、細胞の種類及び保存条件などによって異なってくるが、容器に充填された細胞が生存するのに十分な量であることが必要である。

【0011】

ガス透過性材料としては、この他に多孔質膜があげられる。多孔質膜の場合は、容器内部の液が漏れないようにするために、孔径を所定値以下にする必要がある。好ましい孔径は $1\text{ }\mu\text{m}$ 以下であり、液の漏出防止と容器内への細菌の侵入阻止の点で、 $0.4\text{ }\mu\text{m}$ 以下であることがとくに好ましい。多孔質膜の材料としては、ポリテトラフルオロエチレン、テトラフルオロエチレン-ヘキサフルオロプロピレン、ポリエチレンテレフタレート及びポリプロピレンなどをあげることができる。これらの多孔質膜は容器の側壁全体に使用してもよいし一部に使用してもよい。また、多孔質膜を使用する他の方法として、容器口部に多孔質膜を装着する方法がある。すなわち、容器口部に、多孔質膜を取り付けたキャップを嵌めておけばこの部分でガス交換が行われるので、容器の他の部分はガス不透過性であってもよい。

【0012】

前述したように、本発明の容器に注射器としての機能を持たせると、容器に保存している細胞をそのまま生体内に注入できるので便利である。注射器としては、薬剤などを注射する一般的な注射器と類似の構造にすることもできるが、内部に収納する細胞含有液を押し出すことができるのであれば、従来の注射器とは異なる構造・原理であってもよい。すなわち、一般的な注射器は、外筒、外筒先端に形成された注射針接続部及びピストンから構成されているが、このような構造であってもよいし、容器を蛇腹状にしたり可撓性材料で形成し、内部の細胞含有液を押し出す際には、容器を圧縮したり押し潰すような構成にしてもよい。

【0013】

図1（A）から（C）に、本発明の容器に注射器の機能を持たせた実施例を示す。この実施例では、従来の薬剤注射用の注射器と同様の構造をしており、外筒側面がガス透過性材料で形成されている。すなわち、注射器は外筒1とピストン2から構成されており、ピストン2の先端には、弾性材料からなるガスケット3が取り付けられている。また、外筒1は硬質材料からなる棒体4aと棒体4aの内面に貼付された円筒状のガス透過性シート4bから構成されており、棒体4aには貫孔を形成する複数の窓5が設けられている。したがって、この窓の部分でガス透過性シートが外部に露出しており、ガス交換が行われる。7は注射針接続部6を密封するためのキャップであり、内部の細胞を生体に注入する際にはキャップを外して注射針を装着する。尚、本実施例において示した図1中の窓5の実施形態について、窓5の形状が長方形で、窓5の数が4つの実施形態として示したが、本発明はこの実施形態のみに制限されるものではない。例えば、窓5の形状、寸法、数量について、他の実施形態であっても、本発明の効果は達成可能である。

【0014】

図2は、本発明の第2の実施例を示す図面である。この例では、容器8が蛇腹状に形成されており、その先端に注射針接続部6が設けられている。容器8は全面がガス透過性材料で形成されており、高いガス透過性を達成することができる。この実施例の場合は、図1の実施例に比べて構造が簡単になるので、製造が容易な利点がある。また、保存中にピストンシール部から液漏れをおこす心配もない。内部の細胞を生体に注入するときには、8の後端部を直接指で押すか治具を使用して押せばよい。

【0015】

図3は、本発明の第3の実施例である。この実施例では、細胞は可撓性でガス透過性材料からなる袋状の容器9に収納される。容器9には注射針接続部6が設けられており、細胞を生体内に注入する際には、外筒1内に容器9を収納し、6に注射針を接続した後にピストン2で容器を押して、内部の細胞を押し出す。この例では、ピストンの作用で細胞を押し出すので操作しやすく、細胞は容器内に収納されているので、図1に示す実施例で問題

となるピストンシール部からの液漏れの心配もない。また、外筒とピストンは繰り返して使用することもできる。

【 0 0 1 6 】

図4は、本発明の第4の実施例である。この実施例では第3の実施例と同じように細胞は可撓性の袋10に収納されるが、袋には注射針接続部は設けられておらず、ピストン2を押すと、注射器外筒1の内部に設けられた袋破壊手段11に袋が押し付けられ、袋10が破れて内部の細胞含有液が外筒内に流出し、ピストンで押し出される。袋破壊手段11としては、金属の刃や針のように袋を簡単に破ることができるのが好ましい。

【 0 0 1 7 】

図5は、本発明の第5の実施例である。この実施例では可撓性材料からなる袋12内に細胞を保存するようになっている。容器の一端には注射針接続部6が設けられており、しごき部材13で容器12をしごいて細胞を容器から押し出す。

【 0 0 1 8 】

図6は、ガス透過性の多孔質膜を注射針接続部のキャップに装着してガス透過性の機能を持たせた実施例である。図から分るように、この実施例では、容器は外筒1、ピストン2、ガスケット3及びキャップ12から構成されている。そして、キャップ7には疎水性でガス透過性の膜14が取り付けられており、膜14を通じてガス交換が行われる。

【 0 0 1 9 】

容器に注射器の機能を持たせる場合には、内部の細胞を押し出す際にできるだけ細胞のロスの少ない構造にするのが好ましい。そのためには、容器の形状及び注射器接続部の形状などを工夫すればよい。細胞の残留を少なくできれば、細胞の利用効率が高まるので好ましい。

【 0 0 2 0 】

本発明の容器は、滅菌したものを細胞の保存に使用する。細胞は通常は培地とともに保存するが、生体にそのまま注入するためには、生体に安全な培地を使用する必要がある。たとえば、変形性関節症の患者に軟骨細胞またはその前駆細胞を注入する場合、パーキンソン病の患者の脳に神経細胞またはその前駆細胞を注入する場合及び心臓病の患者に心筋細胞を注入する場合などヒトに細胞を注入する場合には、ウシ血清などの生体由来成分を含まない合成培地や患者自身の血清を使用した培地が好ましい。また、内部に収納する細胞にとくに制限はないが、細胞工学的手法により培養した幹細胞または幹細胞を目的の細胞に分化させた細胞が好ましい。また、これらの細胞を遺伝子工学的手法により改変した遺伝子改変細胞も好ましい。

【 0 0 2 1 】

幹細胞には胚性幹細胞 (embryonic stem cells : ES細胞)、胚性生殖細胞 (embryonic germ cells : EG細胞) および体性幹細胞 (adult stem cells : 成人幹細胞 ; AS細胞) などがあり、分化誘導をかける細胞系列としては、骨細胞、軟骨細胞、筋細胞、心筋細胞、神経細胞、腱細胞、脂肪細胞、臍細胞、肝細胞、皮膚（表皮細胞・線維芽細胞）、血球系細胞などをあげることができる。

【 0 0 2 2 】

本発明の容器は細胞の保存に使用するが、細胞の培養容器として使用し、その後にそのまま細胞を保存してもよい。培養のために足場が必要な場合には、細胞が接着し得る材料から製造したマイクロ粒子を使用するのが好ましい。そのような材料の中でも生体吸収性材料から形成されたものが、生体内に注入後に吸収されて残留しないので好ましい。好適な材料の例としては、ポリ乳酸、ポリグリコール酸、乳酸-グリコール酸共重合体、乳酸-カプロラクトン共重合体、トリメチレンカーボネート、ポリジオキサンノン及びコラーゲンをあげることができる。マイクロ粒子は、多孔質のものが多量の細胞を接着できるので好ましい。

【 0 0 2 3 】

細胞の培養は、公知の方法により実施することができる。すなわち、生体から細胞を分離し、この中から幹細胞を選択的に分離した後、細胞増殖因子または成長因子を添加して培

養する。培養はインキュベータ内で実施するのが好ましい。培養した細胞は、本発明の容器に収納し、保存に適した条件で治療に必要となるまで保存する。保存は低温で行うのが好ましいが、短期間であれば常温または加温下で保存することもできる。

【 0 0 2 4 】

本発明の容器に収納された細胞を生体患部または静脈に注入することにより、変形性関節症、慢性関節リュウマチ、偽関節、進行性筋ジストロフィー症、心筋梗塞、脳卒中、パーキンソン病、脊髄損傷、腱損傷、糖尿病、肝機能障害、消化器機能不全、皮膚損傷、白血病、血液系疾患などに対する治療へ応用される。

【 0 0 2 5 】

【 発明の効果 】

本発明の容器を用いて細胞を保存すれば、従来の方法で必要となる培養容器からの細胞の剥離操作や洗浄操作が不要になる。また、容器に注射器の機能を持たせれば、細胞をそのまま生体に注入できるので、設備の整っていない施設でも容易かつ安全に再生医療を実施することができる。さらに、本発明の容器で細胞の培養も実施すれば、培養・保存・生体注入を簡便な操作で行うことができるので、最も効率的である。そして、容器の少なくとも一部をガス透過性材料で形成すれば、細胞を長期間保存できる利点がある。

【 図面の簡単な説明 】

【図1】(A) 本発明の容器に注射器の機能を持たせた実施例を示す全体斜視図、(B) 本発明の容器の正面図、(C) 本発明の容器の(B)中X-X'線での断面図

【図2】注射器の機能を持たせた本発明の第2の実施例を示す正面図

【図3】注射器の機能を持たせた本発明の第3の実施例を示す部分断面正面図

【図4】注射器の機能を持たせた本発明の第4の実施例を示す部分断面正面図

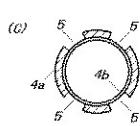
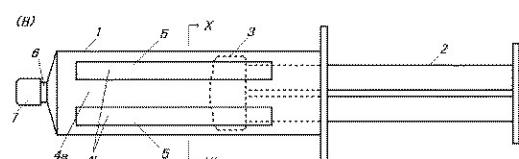
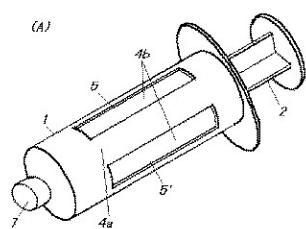
【図5】注射器の機能を持たせた本発明の第5の実施例を示す正面図

【図6】ガス透過性材料を注射針接続部に設けた本発明の実施例を示す正面図

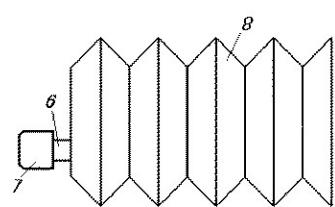
【 符号の説明 】

1. 注射器外筒
2. ピストン
3. ガスケット
- 4 a. 枠体
- 4 b. ガス透過性シート
5. 窓
6. 注射針接続部
7. キャップ[®]
8. 蛇腹状容器
9. 袋状容器1
10. 袋状容器2
11. 袋破壊手段
12. 袋状容器3
13. しごき部材
14. 多孔質膜

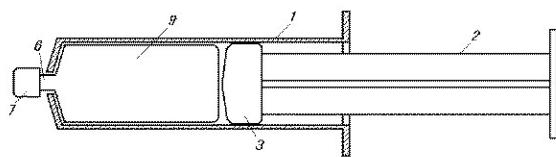
【図1】



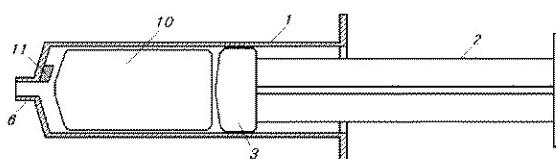
【図2】



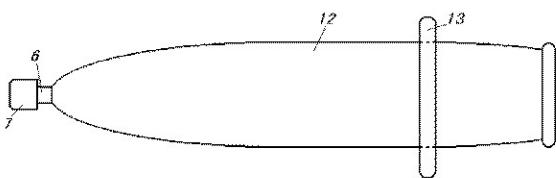
【図3】



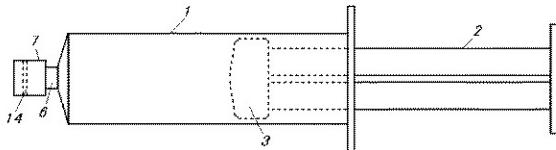
【図4】



【図5】



【図6】



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A61M 5/178
CA/REGISTRY(STN)

JP4511777B2 CELL PRESERVATION CONTAINER

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The wording below is an initial machine translation of the original publication. To generate a version using the latest translation technology, go to the original language text and use Patent Translate.

BACKGROUND OF THE INVENTION 1. Field of the Invention The present invention relates to a method for storing cells therein, and when the cells are needed, immediately removing the cells from the container without using any troublesome operation. The present invention relates to a cell storage container capable of performing the above-mentioned steps. More specifically, the present invention relates to a cell storage container devised so that when cells are used for treatment in regenerative medicine or the like, the cells can be immediately injected into a living body. 2. Description of the Related Art In recent years, due to advances in cell biology and cell engineering, research on regenerative medicine in which cells (stem cells) having pluripotency and self-renewal ability extracted from a living body are transplanted into a patient and treated. Is progressing rapidly. This is a treatment method for repairing and recovering a target tissue (organ) by transplanting a stem cell obtained by inducing differentiation into a target cell into a deficient part of a living tissue or a site responsible for a disease. At present, it is still in the stage of basic research, but some clinical trials have begun. In such a case, generally, undifferentiated cells or differentiated cells obtained by culturing cells alone or in a dedicated container in the presence of a scaffold are transplanted to a target site by surgery. [0003] The above-described cell transplantation method requires a surgical operation, and therefore places a heavy burden on a patient. Therefore, a method of directly injecting cells into a living body has been studied. In particular, injection of chondrocytes or their precursor cells into joints, injection of nerve cells or their precursor cells into the brain, injection of cardiomyocytes or their precursor cells into the heart, etc. can be effective treatments. In the case of performing such treatment, in the conventional method, cells that have proliferated and adhered to the container wall are treated with trypsin, EDTA, or the like to be detached, and after a step such as washing, a predetermined amount is collected in a syringe. It will be injected into the living body. [0004] However, such a method is not only troublesome in many operation steps but also requires a series of operations to be performed by a skilled person in a clean environment so as not to cause contamination from the surroundings. For this reason, it is difficult to carry out cell transplantation treatment in facilities other than well-equipped facilities. [0005] An object of the present invention is to provide a medical device that can easily carry out cell transplantation treatment. That is, the present invention provides a medical device that allows anyone to easily store and inject cells into a living body. According to the present invention, there is provided an outer cylinder provided with a cell accommodating portion capable of accommodating a cell-containing liquid therein, and a slidably inserted cell inside the outer cylinder. A syringe-specific cell storage container having a piston capable of increasing and decreasing the pressing force so that the volume can be varied, and the inner surface of the cell container is made of a material selected from the group consisting of polyacrylic acid and polymethacrylic acid. The above-mentioned problem was solved by storing the grown cells in a container formed of a material bound or coated with the compound. That is, the present invention is a cell storage container in which the inner surface of the cell accommodating portion is formed of a material such as polyacrylic acid and polymethacrylic acid to which cells are difficult to adhere. By forming the inner surface of the container with a material to which the cells do not easily adhere, the cells do not adhere to the container wall surface during storage, and the operation of peeling the cells from the container wall surface becomes unnecessary. The expression "the cells are hard to adhere" means that the cells do not adhere at all during the storage of the cells, or that they adhere only to such an extent that they are easily peeled off even if they adhere. Further, the present invention relates to a cell storage container of a syringe specification, and it is possible to easily transfer a cell-containing liquid into and out of the cell storage container by a relatively simple operation. **BEST MODE FOR CARRYING OUT THE INVENTION** The cell storage container of the present invention has an inner surface formed of a material to which cells are hardly adhered. However, if the container has a function of a syringe, it can be directly injected into a living body. It is convenient because you can do it. In addition, it is preferable to form a part or the whole of the container with a gas-permeable material, since oxygen and carbon dioxide necessary for survival of cells can be permeated even in a sealed state, and the cells can be stored for a long period of time. Examples Examples of the material which is difficult to adhere to cells used in the present invention include those whose surface is formed of a hydrophilic material or a hydrophobic material and those whose surface has a negative charge. The hydrophilic material preferably has a contact angle with water of 50 degrees or less, and the hydrophobic material preferably has a contact angle with water of 100 degrees or more. Examples of preferred hydrophilic materials include acrylamide polymers, methacrylamide polymers, polyacrylic acid, polymethacrylic acid, polyvinyl alcohol, polyethylene glycol, polyvinylpyrrolidone, cellulose, dextran,

hyaluronic acid, glycosumming glycans, proteoglycans, Materials in which carrageenan, protein, and the like are bonded to the surface of the base material by a method such as graft copolymerization or chemical reaction, or the surface is coated. Examples of the hydrophobic material include fluororesins such as polytetrafluoroethylene and tetrafluoroethylene-hexafluoropropylene copolymer and silicone resins. Examples of the material having a negative charge on the surface include materials having polyacrylic acid, polymethacrylic acid, styrenesulfonic acid, alginic acid, heparin, heparan sulfate, chondroitin sulfate, or dermatan bonded to the surface. Among these, a material having a carboxyl group on the surface is particularly preferable. Particularly preferred materials include polyacrylic acid and polymethacrylic acid. The reason is that the material has both the cell non-adhesive effect as the above-mentioned hydrophilic material and the cell non-adhesive effect due to negative charge. Further, it is preferable that the surface of the material is smooth because it has excellent non-adhesiveness of cells. In the present invention, a method for forming a part or the whole of a container with a gas permeable material includes a method for forming a part or the whole of a side wall of the container with a non-porous material having good gas permeability. A method of attaching a porous membrane to a part of a container so that gas exchange is performed can be given. Materials having good gas permeability include silicone resin, poly (4-methylpentene) 1, polyisoprene, polybutadiene, ethylene-vinyl acetate copolymer, low-density polyethylene, polystyrene and the like. These materials have relatively good gas permeability among plastic materials, but since the gas permeability decreases as the thickness increases, it is usually preferably 200 μm or less, and preferably 100 μm or less. Particularly preferred. In the present invention, the entire container may be formed of such a material, or only a part thereof may be formed of such a material. The required gas permeability varies depending on the surface area of the container, the amount of packed cells, the type of cells, storage conditions, and the like, but it is necessary that the amount of cells filled in the container be sufficient to survive. [0011] As the gas permeable material, a porous membrane may be used. In the case of a porous membrane, the pore diameter needs to be smaller than a predetermined value in order to prevent the liquid inside the container from leaking. The preferred pore diameter is 1 μm or less, and particularly preferably 0.4 μm or less from the viewpoint of preventing leakage of liquid and preventing bacteria from entering the container. Examples of the material of the porous film include polytetrafluoroethylene, tetrafluoroethylene-hexafluoropropylene, polyethylene terephthalate, and polypropylene. These porous membranes may be used on the entire side wall of the container or on a part thereof. Another method of using a porous membrane is to attach the porous membrane to the container mouth. That is, if a cap provided with a porous membrane is fitted in the container mouth, gas exchange is performed in this portion, and the other portion of the container may be gas-impermeable. As described above, when the container of the present invention is provided with a function as a syringe, it is convenient that cells stored in the container can be directly injected into a living body. The syringe can have a structure similar to that of a general syringe for injecting medicines, etc., but if the cell-containing liquid stored inside can be pushed out, it has a different structure and principle from the conventional syringe. There may be. That is, a general syringe is composed of an outer cylinder, a syringe needle connection portion formed at the tip of the outer cylinder, and a piston. However, such a structure may be used, or the container may be made bellows or flexible. When extruding the cell-containing liquid inside the container, the container may be compressed or crushed. FIGS. 1A to 1C show an embodiment in which the container of the present invention has a function of a syringe. In this embodiment, the structure is the same as that of a conventional syringe for drug injection, and the outer cylinder side surface is formed of a gas permeable material. That is, the syringe includes an outer cylinder 1 and a piston 2, and a gasket 3 made of an elastic material is attached to a tip of the piston 2. The outer cylinder 1 is composed of a frame 4a made of a hard material and a cylindrical gas permeable sheet 4b adhered to the inner surface of the frame 4a. The frame 4a has a plurality of windows forming through holes 5 are provided. Therefore, the gas permeable sheet is exposed to the outside at this window portion, and gas exchange is performed. Reference numeral 7 denotes a cap for sealing the injection needle connection portion 6. When injecting the cells inside the living body, the cap is removed and the injection needle is attached. In the embodiment of the window 5 in FIG. 1 shown in this embodiment, the shape of the window 5 is rectangular and the number of the windows 5 is four, but the present invention is limited to this embodiment only. It is not done. For example, the effects of the present invention can be achieved even in other embodiments with respect to the shape, dimensions, and number of the windows 5. FIG. 2 is a drawing showing a second embodiment of the present invention. In this example, the container 8 is formed in a bellows shape, and the injection needle connection portion 6 is provided at the tip thereof. The entire surface of the container 8 is formed of a gas permeable material, and high gas permeability can be achieved. In the case of this embodiment, the structure is simpler than that of the embodiment of FIG. In addition, there is no risk of liquid leakage from the piston seal during storage. When injecting the cells inside the living body, the rear end of 8 may be pressed directly with a finger or by using a jig. FIG. 3 shows a third embodiment of the present invention. In this embodiment, the cells are stored in a bag-shaped container 9 made of a flexible and gas-permeable material. The container 9 is provided with an injection needle connection portion 6. When injecting cells into a living body, the container 9 is housed in the outer cylinder 1, the injection needle is connected to 6, and then the container is connected with the piston 2. Press to push out the cells inside.

In this example, the cells are pushed out by the action of the piston, so that the operation is easy. Since the cells are stored in the container, there is no need to worry about liquid leakage from the piston seal portion which is a problem in the embodiment shown in FIG. Further, the outer cylinder and the piston can be used repeatedly. FIG. 4 shows a fourth embodiment of the present invention. In this embodiment, the cells are stored in a flexible bag 10 as in the third embodiment, but the bag is not provided with a syringe needle connection portion. The bag is pressed against the bag breaking means 11 provided inside 1, the bag 10 is torn, and the cell-containing liquid inside flows out into the outer cylinder and is pushed out by the piston. The bag breaking means 11 is preferably one that can easily break the bag, such as a metal blade or a needle. FIG. 5 shows a fifth embodiment of the present invention. In this embodiment, cells are stored in a bag 12 made of a flexible material. An injection needle connection portion 6 is provided at one end of the container, and the cells are pushed out of the container by squeezing the container 12 with an ironing member 13. FIG. 6 shows an embodiment in which a gas permeable porous membrane is attached to the cap of the connection portion of the injection needle to have a gas permeable function. As can be seen, in this embodiment, the container comprises an outer cylinder 1, a piston 2, a gasket 3 and a cap 12. Further, a hydrophobic gas-permeable membrane 14 is attached to the cap 7, and gas exchange is performed through the membrane 14. In the case where the container has the function of a syringe, it is preferable to adopt a structure in which the loss of cells is as small as possible when pushing out the cells inside. For that purpose, the shape of the container and the shape of the syringe connection may be devised. It is preferable that the residual cells can be reduced because the efficiency of cell utilization is increased. The container of the present invention comprises: Use the sterile one for cell preservation. The cells are usually stored together with the medium, but injecting them directly into the living body requires the use of a medium that is safe for the living body. For example, when injecting chondrocytes or its precursor cells into patients with osteoarthritis, when injecting neurons or their precursor cells into the brain of patients with Parkinson's disease, and when injecting cardiomyocytes into patients with heart disease. When cells are injected into humans, a synthetic medium containing no biological components such as bovine serum or a medium using the patient's own serum is preferred. There is no particular limitation on the cells to be housed therein, but stem cells cultured by cell engineering techniques or cells obtained by differentiating stem cells into target cells are preferable. Genetically modified cells obtained by modifying these cells by genetic engineering techniques are also preferred. The stem cells include embryonic stem cells (embryonic stem cells: ES cells), embryonic germ cells (embryonic germ cells: EG cells), and somatic stem cells (adult stem cells: adult stem cells; AS cells). Cell lines to be induced include bone cells, chondrocytes, muscle cells, cardiomyocytes, nerve cells, tendon cells, fat cells, pancreatic cells, hepatocytes, skin (epidermal cells / fibroblasts), blood cells, etc. I can give it. Although the container of the present invention is used for storing cells, it may be used as a cell culture container, and then the cells may be stored as they are. If a scaffold is required for culturing, it is preferable to use microparticles made from a material to which cells can adhere. Among such materials, those formed of a bioabsorbable material are preferable because they are absorbed and do not remain after being injected into a living body. Examples of suitable materials include polylactic acid, polyglycolic acid, lactic acid-glycolic acid copolymer, lactic acid-caprolactone copolymer, trimethylene carbonate, polydioxanone, and collagen. Microparticles are preferred because they are porous and can adhere large amounts of cells. The culturing of the cells can be performed by a known method. That is, cells are separated from a living body, and stem cells are selectively separated therefrom, and then cultured by adding a cell growth factor or a growth factor. The cultivation is preferably performed in an incubator. The cultured cells are stored in the container of the present invention and stored under conditions suitable for storage until needed for treatment. Storage is preferably performed at a low temperature, but for a short period of time, storage can be performed at room temperature or under heating. By injecting the cells contained in the container of the present invention into a diseased part of a living body or a vein, osteoarthritis, rheumatoid arthritis, pseudoarthritis, progressive muscular dystrophy, myocardial infarction, stroke, Parkinson's disease, spinal cord injury It is applied to the treatment of tendon damage, diabetes, liver dysfunction, digestive dysfunction, skin damage, leukemia, blood system disease and the like. According to the present invention, when cells are stored using the container of the present invention, the operation of detaching cells from the culture container and the operation of washing, which are required in the conventional method, become unnecessary. In addition, if the container has the function of a syringe, cells can be directly injected into a living body, so that regenerative medicine can be easily and safely performed even in a facility without facilities. Furthermore, culturing cells in the container of the present invention is the most efficient because culturing, preserving, and injecting a living body can be performed by simple operations. If at least a part of the container is formed of a gas-permeable material, there is an advantage that cells can be stored for a long time.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 (A) Overall perspective view showing an embodiment in which the container of the present invention has a function of a syringe, (B) Front view of the container of the present invention, (C) The present invention FIG. 2 is a cross-sectional view of the container taken along line XX' in FIG. 2B. FIG. 2 is a front view showing a second embodiment of the present invention having the function of a syringe. FIG. 3 is having the function of a syringe. FIG. 4 is a partial cross-sectional front view showing a third embodiment of the present invention. FIG. 4 is a partial cross-sectional front view showing a

fourth embodiment of the present invention having a syringe function. FIG. 6 is a front view showing a fifth embodiment of the present invention. FIG. 6 is a front view showing an embodiment of the present invention in which a gas permeable material is provided at a connection portion of a syringe needle. 1. syringe barrel Piston 3. Gasket 4a. Frame 4b. Gas permeable sheet5. Window6. Injection needle connection 7. Cap8. 8. bellows-shaped container Bag-shaped container 110. Bag-shaped container 211. Bag breaking means 12. Bag-shaped container 313. Ironing member 14. Porous membrane

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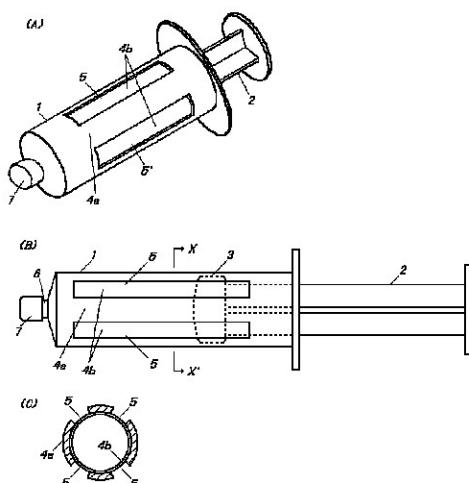
(54) 【発明の名称】細胞保存容器

(57) 【要約】

【課題】本発明の目的は、細胞移植治療を簡便に実施することができる医療器具を提供することにある。すなわち、細胞の保存及び生体への注入を誰でも容易に行うことができる医療器具を提供するものである。

【解決手段】上記課題を解決するため、本発明の細胞保存容器は容器の内面が、細胞の接着しにくい材料、例えば親水性材料あるいは疎水性材料が結合または被覆された材料で形成されてなるため、従来の細胞の剥離操作や洗浄操作が不要となる。また、容器の少なくとも一部がガス透過性の材料で形成されており、細胞の生存に必要なガス交換が可能になり、細胞の長期保存が可能となる。また、注射器様の形態をなすことにより容易に注入操作を可能とする。以上の構成により、培養・保存・生体注入を簡便な操作で行うことができる細胞保存容器を提供することが可能となる。

【選択図】 図1



【特許請求の範囲】**【請求項1】**

容器の内面が、細胞の接着しにくい材料で形成されてなる細胞保存容器。

【請求項2】

細胞の接着しにくい材料が、表面に親水性材料が結合または被覆された材料である請求項1記載の細胞保存容器。

【請求項3】

親水性材料が、アクリルアミド系重合体、メタクリルアミド系重合体、ポリアクリル酸、ポリメタクリル酸、ポリビニルアルコール、ポリエチレンジリコール、ポリビニルピロリドン、セルロース、デキストラン、ヒアルロン酸、グリコサミングリカン、プロテオグリカン、カラギーナン及びタンパク質からなる群より選ばれた材料である請求項2記載の細胞保存容器。

【請求項4】

細胞の接着しにくい材料が、表面に負電荷を有する材料である請求項1記載の細胞保存容器。

【請求項5】

表面に負電荷を有する材料が、ポリアクリル酸、ポリメタクリル酸、スチレンスルホン酸、アルギン酸、ヘパリン、ヘパラン硫酸、コンドロイチン硫酸またはデルマタンが表面に結合した材料である請求項4記載の細胞保存容器。

【請求項6】

細胞の接着しにくい材料が、表面に疎水性材料が結合または被覆された材料である請求項1記載の細胞保存容器。

【請求項7】

疎水性材料が、フッ素樹脂またはシリコーン樹脂である請求項6記載の細胞保存容器。

【請求項8】

容器が、注射器としての機能を有する請求項1～7の何れかの項に記載の細胞保存容器。

【請求項9】

容器が蛇腹状に形成されてなり、蛇腹部分を圧縮することによって細胞を含む内溶液が押し出され、注射器としての機能を発揮する請求項8記載の細胞保存容器。

【請求項10】

容器が可撓性材料から形成されてなり、可撓性部分を押し潰すことによって細胞を含む内溶液が押し出され、注射器としての機能を発揮する請求項8記載の細胞保存容器。

【請求項11】

容器が、細胞を収納する袋と、該袋を収納可能な注射器と、注射器に設けられた袋破壊手段からなる請求項8記載の細胞保存容器。

【請求項12】

容器が可撓性材料から形成されてなり、可撓性部分をしごくことによって細胞を含む内溶液が押し出され、注射器としての機能を発揮する請求項8記載の細胞保存容器。

【請求項13】

容器の一部または全部が、ガス透過性材料で形成されてなる請求項1～12の何れかの項に記載の細胞保存容器。

【請求項14】

ガス透過性材料が、ガス透過性の良好な合成樹脂で形成された非多孔質のシートである請求項13記載の細胞保存容器。

【請求項15】

ガス透過性の良好な合成樹脂が、シリコーン樹脂、ポリ4メチルペンテン1、ポリイソブレン、ポリブタジエン、エチレン酢酸ビニル共重合体、低密度ポリエチレンまたはポリスチレンである請求項14記載の細胞保存容器。

【請求項16】

ガス透過性材料が、疎水性材料からなる多孔質膜である請求項1～3記載の細胞保存容器。

【請求項17】

容器側壁の少なくとも一部が多孔質膜で形成されてなる請求項1～6記載の細胞保存容器。

【請求項18】

多孔質膜が容器口部に装着されたキャップに取り付けられてなる請求項1～6記載の細胞保存容器。

【請求項19】

多孔質膜の孔径が1μm以下である請求項1～6～18の何れかの項に記載の細胞保存容器。

【請求項20】

多孔質膜の孔径が0.4μm以下である請求項1～6～18の何れかの項に記載の細胞保存容器。

【請求項21】

疎水性材料が、ポリテトラフルオロエチレン、テトラフルオロエチレン-ヘキサフルオロプロピレン共重合体、ポリエチレンテレフタレートまたはポリプロピレンである請求項1～20の何れかの項に記載の細胞保存容器。

【請求項22】

請求項1～21の何れかの項に記載の容器に細胞含有液が充填されてなる細胞製品。

【請求項23】

細胞製品が、治療用である請求項2～2記載の細胞製品。

【請求項24】

細胞が胚性幹細胞または体性幹細胞である請求項2～2または2～3記載の細胞製品。

【請求項25】

細胞が骨細胞である請求項2～2または2～3記載の細胞製品。

【請求項26】

細胞が軟骨細胞である請求項2～2または2～3記載の細胞製品。

【請求項27】

細胞が筋細胞である請求項2～2または2～3記載の細胞製品。

【請求項28】

細胞が心筋細胞である請求項2～2または2～3記載の細胞製品。

【請求項29】

細胞が神経細胞である請求項2～2または2～3記載の細胞製品。

【請求項30】

細胞が腱細胞である請求項2～2または2～3記載の細胞製品。

【請求項31】

細胞が脂肪細胞である請求項2～2または2～3記載の細胞製品。

【請求項32】

細胞が臍細胞である請求項2～2または2～3記載の細胞製品。

【請求項33】

細胞が肝細胞である請求項2～2または2～3記載の細胞製品。

【請求項34】

細胞が皮膚細胞である請求項2～2または2～3記載の細胞製品。

【請求項35】

細胞が血球系細胞である請求項2～2または2～3記載の細胞製品。

【発明の詳細な説明】

【0001】

【発明の属する技術分野】

本発明は、内部に細胞を保存することができ、細胞が必要になった時には、面倒な操作をすることなく、ただちに容器から細胞を取り出して使用することができる細胞保存容器に関する。さらに詳しくは、再生医療などにおいて治療に細胞を使用する際に、そのままぐに生体に注入できるように工夫した細胞保存容器に関する。

【0002】**【従来の技術】**

近年、細胞生物学や細胞工学の進歩により、生体から取り出した多分化能と自己複製能を持つ細胞（幹細胞）を患者に移植して治療を行う再生医療の研究が急速に進められている。これは標的細胞へ分化誘導をかけた幹細胞を生体組織の欠損部や疾患の責任病巣部位へ移植して目的組織（臓器）を修復・回復させる治療法である。現在はまだ基礎的研究の段階であるが、臨床試験も一部で試みられ始めている。このような場合、一般には、細胞を単独または足場の存在下に専用の容器内で培養した未分化な細胞または分化した細胞を、外科手術によって目的部位に移植する。

【0003】**【発明が解決しようとする課題】**

上述したような細胞の移植方法では外科手術が必要となるため、患者への負担が大きい。そこで、細胞を直接生体内に注入する方法が検討されている。とくに関節への軟骨細胞またはその前駆細胞の注入、脳への神経細胞またはその前駆細胞の注入、心臓への心筋細胞またはその前駆細胞の注入などは、有力な治療法となり得る。このような治療を実施する場合、従来の方法では、増殖して容器壁に接着している細胞をトリプシンやE D T Aなどで処理して剥離し、洗浄などの工程を経て所定量を注射器に採取し生体に注入することになる。

【0004】

しかし、このような方法では操作の工程が多く面倒であるだけでなく、一連の操作は周囲からの汚染が起こらないようにクリーンな環境下で熟練した者が行う必要がある。このため、設備の整った施設以外では細胞移植治療を実施するのが難しい。

【0005】

本発明の目的は、細胞移植治療を簡便に実施することができる医療器具を提供することにある。すなわち、細胞の保存及び生体への注入を誰でも容易に行うことができる医療器具を提供するものである。

【0006】**【課題を解決するための手段】**

本発明においては、増殖させた細胞を、内面を細胞の接着しにくい材料で形成した容器に保存することにより、上述した課題を解決した。すなわち本発明は、容器の内面が、細胞の接着しにくい材料で形成されてなる細胞保存容器である。容器内面を細胞の接着しにくい材料で形成することにより、保存中に細胞が容器壁面に接着することがなくなり、細胞を容器壁面から剥離する操作が不用になる。なお、「細胞が接着しにくい」とは、細胞保存中に細胞が全く接着しないかあるいは接着しても簡単に剥がれる程度にしか接着しないことを意味する。

【0007】**【発明の実施の形態】**

本発明の細胞保存容器は、内面が細胞の接着しにくい材料で形成されているが、容器を注射器の機能を有するものにしておけば、そのまま生体に注入することができる所以便利である。また、容器の一部または全部をガス透過性の材料で形成すれば、密封状態でも細胞の生存に必要な酸素や炭酸ガスが透過できるようになり、細胞の長期保存が可能になるので好ましい。

【0008】**【実施例】**

本発明において使用する細胞の接着しにくい材料としては、表面が親水性材料または疎水性材料で形成されたもの及び表面に負電荷を有する材料をあげることができる。親水性材料としては対水接触角が50度以下のものが好ましく、疎水性材料としては対水接触角が100度以上のものが好ましい。好ましい親水性材料の例としては、アクリルアミド系重合体、メタクリルアミド系重合体、ポリアクリル酸、ポリメタクリル酸、ポリビニルアルコール、ポリエチレングリコール、ポリビニルピロリドン、セルロース、デキストラン、

ヒアルロン酸、グリコサミングリカン、プロテオグリカン、カラギーナン及びタンパク質などを基材の表面にグラフト共重合や化学反応などの方法で結合するか表面に被覆した材料をあげることができる。また、疎水性材料としては、ポリテトラフルオロエチレン、テトラフルオロエチレン-ヘキサフルオロプロピレン共重合体などのフッ素樹脂及びシリコーン樹脂をあげることができる。また、表面に負電荷を有する材料としては、ポリアクリル酸、ポリメタクリル酸、スチレンスルホン酸、アルギン酸、ヘパリン、ヘパラン硫酸、コンドロイチン硫酸またはデルマタンを表面に結合した材料をあげることができる。これらの中でも、表面にカルボキシル基を有する材料がとくに好ましい。また、材料の表面は平滑な方が細胞の非接着性に優れているので好ましい。

【 0 0 0 9 】

本発明において、容器の一部または全部をガス透過性材料で形成する方法としては、容器の側壁の一部または全部をガス透過性の良好な非多孔質材料で形成する方法と、容器の一部に多孔質膜を装着してガス交換が行われるようにする方法をあげることができる。

【 0 0 1 0 】

ガス透過性の良好な材料としては、シリコーン樹脂、ポリ4メチルペンテン1、ポリイソブレン、ポリブタジエン、エチレン酢酸ビニル共重合体、低密度ポリエチレン及びポリスチレンなどをあげることができる。これらの材料は、プラスチック材料の中では比較的良好なガス透過性を有しているが、厚さが厚くなるとガス透過性が低下するので、通常は200μm以下であることが好ましく、100μm以下がとくに好ましい。本発明においては、容器全体をこのような材料で形成することもできるし、一部だけをそのような材料で形成してもよい。必要なガス透過度は、容器の表面積、細胞の充填量、細胞の種類及び保存条件などによって異なってくるが、容器に充填された細胞が生存するのに十分な量であることが必要である。

【 0 0 1 1 】

ガス透過性材料としては、この他に多孔質膜があげられる。多孔質膜の場合は、容器内部の液が漏れないようにするために、孔径を所定値以下にする必要がある。好ましい孔径は1μm以下であり、液の漏出防止と容器内への細菌の侵入阻止の点で、0.4μm以下であることがとくに好ましい。多孔質膜の材料としては、ポリテトラフルオロエチレン、テトラフルオロエチレン-ヘキサフルオロプロピレン、ポリエチレンテレフタレート及びポリプロピレンなどをあげることができる。これらの多孔質膜は容器の側壁全体に使用してもよいし一部に使用してもよい。また、多孔質膜を使用する他の方法として、容器口部に多孔質膜を装着する方法がある。すなわち、容器口部に、多孔質膜を取り付けたキャップを嵌めておけばこの部分でガス交換が行われるので、容器の他の部分はガス不透過性であってもよい。

【 0 0 1 2 】

前述したように、本発明の容器に注射器としての機能を持たせると、容器に保存している細胞をそのまま生体内に注入できるので便利である。注射器としては、薬剤などを注射する一般的な注射器と類似の構造にすることもできるが、内部に収納する細胞含有液を押し出すことができるのであれば、従来の注射器とは異なる構造・原理であってもよい。すなわち、一般的な注射器は、外筒、外筒先端に形成された注射針接続部及びピストンから構成されているが、このような構造であってもよいし、容器を蛇腹状にしたり可撓性材料で形成し、内部の細胞含有液を押し出す際には、容器を圧縮したり押し潰すような構成にしてもよい。

【 0 0 1 3 】

図1 (A) から (C) に、本発明の容器に注射器の機能を持たせた実施例を示す。この実施例では、従来の薬剤注射用の注射器と同様の構造をしており、外筒側面がガス透過性材料で形成されている。すなわち、注射器は外筒1とピストン2から構成されており、ピストン2の先端には、弾性材料からなるガスケット3が取り付けられている。また、外筒1は硬質材料からなる棒体4aと棒体4aの内面に貼付された円筒状のガス透過性シート4bから構成されており、棒体4aには貫孔を形成する複数の窓5が設けられている。した

がって、この窓の部分でガス透過性シートが外部に露出しており、ガス交換が行われる。7は注射針接続部6を密封するためのキャップであり、内部の細胞を生体に注入する際にキャップを外して注射針を装着する。尚、本実施例において示した図1中の窓5の実施形態について、窓5の形状が長方形で、窓5の数が4つの実施形態として示したが、本発明はこの実施形態のみに制限されるものではない。例えば、窓5の形状、寸法、数量について、他の実施形態であっても、本発明の効果は達成可能である。

【0014】

図2は、本発明の第2の実施例を示す図面である。この例では、容器8が蛇腹状に形成されており、その先端に注射針接続部6が設けられている。容器8は全面がガス透過性材料で形成されており、高いガス透過性を達成することができる。この実施例の場合は、図1の実施例に比べて構造が簡単になるので、製造が容易な利点がある。また、保存中にピストンシール部から液漏れをおこす心配もない。内部の細胞を生体に注入するときには、8の後端部を直接指で押すか治具を使用して押せばよい。

【0015】

図3は、本発明の第3の実施例である。この実施例では、細胞は可撓性でガス透過性材料からなる袋状の容器9に収納される。容器9には注射針接続部6が設けられており、細胞を生体内に注入する際には、外筒1内に容器9を収納し、6に注射針を接続した後にピストン2で容器を押して、内部の細胞を押し出す。この例では、ピストンの作用で細胞を押し出すので操作しやすく、細胞は容器内に収納されているので、図1に示す実施例で問題となるピストンシール部からの液漏れの心配もない。また、外筒とピストンは繰り返して使用することもできる。

【0016】

図4は、本発明の第4の実施例である。この実施例では第3の実施例と同じように細胞は可撓性の袋10に収納されるが、袋には注射針接続部は設けられておらず、ピストン2を押すと、注射器外筒1の内部に設けられた袋破壊手段11に袋が押しつけられ、袋10が破れて内部の細胞含有液が外筒内に流出し、ピストンで押し出される。袋破壊手段11としては、金属の刃や針のように袋を簡単に破ることができるものが好ましい。

【0017】

図5は、本発明の第5の実施例である。この実施例では可撓性材料からなる袋12内に細胞を保存するようになっている。容器の一端には注射針接続部6が設けられており、しごき部材13で容器12をしごいて細胞を容器から押し出す。

【0018】

図6は、ガス透過性の多孔質膜を注射針接続部のキャップに装着してガス透過性の機能を持たせた実施例である。図から分るように、この実施例では、容器は外筒1、ピストン2、ガスケット3及びキャップ12から構成されている。そして、キャップ7には疎水性でガス透過性の膜14が取り付けられており、膜14を通じてガス交換が行われる。

【0019】

容器に注射器の機能を持たせる場合には、内部の細胞を押し出す際にできるだけ細胞の口の少ない構造にするのが好ましい。そのためには、容器の形状及び注射器接続部の形状などを工夫すればよい。細胞の残留を少なくできれば、細胞の利用効率が高まるので好ましい。

【0020】

本発明の容器は、滅菌したものを細胞の保存に使用する。細胞は通常は培地とともに保存するが、生体にそのまま注入するためには、生体に安全な培地を使用する必要がある。たとえば、変形性関節症の患者に軟骨細胞またはその前駆細胞を注入する場合、パーキンソン病の患者の脳に神経細胞またはその前駆細胞を注入する場合及び心臓病の患者に心筋細胞を注入する場合などヒトに細胞を注入する場合には、ウシ血清などの生体由来成分を含まない合成培地や患者自身の血清を使用した培地が好ましい。また、内部に収納する細胞にとくに制限はないが、細胞工学的手法により培養した幹細胞または幹細胞を目的の細胞に分化させた細胞が好ましい。また、これらの細胞を遺伝子工学的手法により改変した遺

伝子改変細胞も好ましい。

【0021】

幹細胞には胚性幹細胞 (embryonic stem cells : ES細胞)、胚性生殖細胞 (embryonic germ cells : EG細胞) および体性幹細胞 (adult stem cells : 成人幹細胞 ; AS細胞) などがあり、分化誘導をかける細胞系列としては、骨細胞、軟骨細胞、筋細胞、心筋細胞、神経細胞、腱細胞、脂肪細胞、臍細胞、肝細胞、皮膚（表皮細胞・線維芽細胞）、血球系細胞などをあげることができる。

【0022】

本発明の容器は細胞の保存に使用するが、細胞の培養容器として使用し、その後にそのまま細胞を保存してもよい。培養のために足場が必要な場合には、細胞が接着し得る材料から製造したマイクロ粒子を使用するのが好ましい。そのような材料の中でも生体吸収性材料から形成されたものが、生体内に注入後に吸収されて残留しないので好ましい。好適な材料の例としては、ポリ乳酸、ポリグリコール酸、乳酸-グリコール酸共重合体、乳酸-カプロラクトン共重合体、トリメチレンカーボネート、ポリジオキサン及びコラーゲンをあげることができる。マイクロ粒子は、多孔質のものが多量の細胞を接着できるので好ましい。

【0023】

細胞の培養は、公知の方法により実施することができる。すなわち、生体から細胞を分離し、この中から幹細胞を選択的に分離した後、細胞増殖因子または成長因子を添加して培養する。培養はインキュベータ内で実施するのが好ましい。培養した細胞は、本発明の容器に収納し、保存に適した条件で治療に必要となるまで保存する。保存は低温で行うのが好ましいが、短期間であれば常温または加温下で保存することもできる。

【0024】

本発明の容器に収納された細胞を生体患部または静脈に注入することにより、変形性関節症、慢性関節リュウマチ、偽関節、進行性筋ジストロフィー症、心筋梗塞、脳卒中、パーキンソン病、脊髄損傷、腱損傷、糖尿病、肝機能障害、消化器機能不全、皮膚損傷、白血病、血液系疾患などに対する治療へ応用される。

【0025】

【発明の効果】

本発明の容器を用いて細胞を保存すれば、従来の方法で必要となる培養容器からの細胞の剥離操作や洗浄操作が不要になる。また、容器に注射器の機能を持たせれば、細胞をそのまま生体に注入できるので、設備の整っていない施設でも容易かつ安全に再生医療を実施することができる。さらに、本発明の容器で細胞の培養も実施すれば、培養・保存・生体注入を簡便な操作で行うことができるので、最も効率的である。そして、容器の少なくとも一部をガス透過性材料で形成すれば、細胞を長期間保存できる利点がある。

【図面の簡単な説明】

【図1】(A) 本発明の容器に注射器の機能を持たせた実施例を示す全体斜視図、(B) 本発明の容器の正面図、(C) 本発明の容器の(B)中X-X'線での断面図

【図2】注射器の機能を持たせた本発明の第2の実施例を示す正面図

【図3】注射器の機能を持たせた本発明の第3の実施例を示す部分断面正面図

【図4】注射器の機能を持たせた本発明の第4の実施例を示す部分断面正面図

【図5】注射器の機能を持たせた本発明の第5の実施例を示す正面図

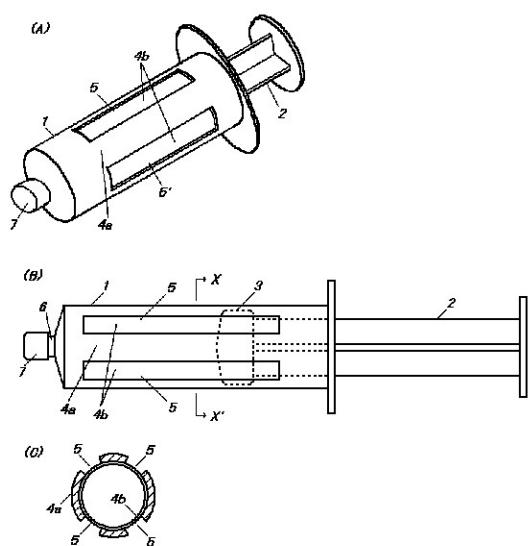
【図6】ガス透過性材料を注射針接続部に設けた本発明の実施例を示す正面図

【符号の説明】

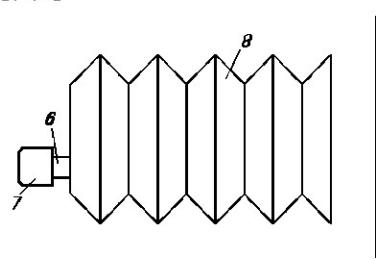
1. 注射器外筒
2. ピストン
3. ガスケット
- 4 a. 柄体
- 4 b. ガス透過性シート

5. 窓
6. 注射針接続部
7. キャップ
8. 蛇腹状容器
9. 袋状容器1
10. 袋状容器2
11. 袋破壊手段
12. 袋状容器3
13. しごき部材
14. 多孔質膜

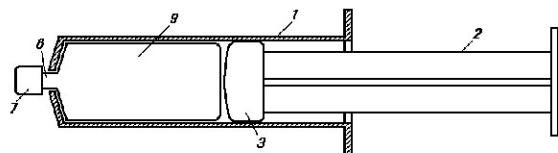
【図1】



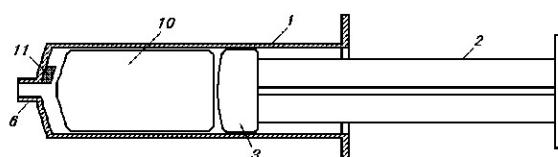
【図2】



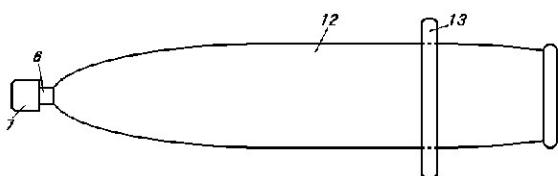
【図3】



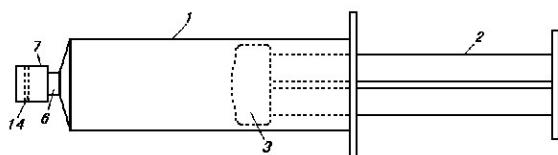
【図4】



【図5】



【図6】



(51)Int.Cl.⁷// C 1 2 N 1/04
C 1 2 N 5/06

F I

C 1 2 N 5/00
C 1 2 N 1/04

E

テーマコード(参考)

JP2004018504A CELL PRESERVATION CONTAINER

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The wording below is an initial machine translation of the original publication. To generate a version using the latest translation technology, go to the original language text and use Patent Translate.

BACKGROUND OF THE INVENTION 1. Field of the Invention The present invention can store cells inside, and when cells are needed, immediately take out the cells from the container and use them without troublesome operations. The present invention relates to a cell storage container. More specifically, the present invention relates to a cell storage container devised so that it can be immediately injected into a living body when cells are used for treatment in regenerative medicine or the like. 2. Description of the Related Art Recent advances in cell biology and cell engineering have led to research on regenerative medicine in which cells (stem cells) with multipotency and self-replication ability removed from living bodies are transplanted into patients for treatment. Is progressing rapidly. This is a treatment method for repairing / recovering a target tissue (organ) by transplanting a stem cell that has been induced to differentiate into a target cell to a defective part of a living tissue or a responsible lesion site of a disease. Although it is still in the basic research stage, some clinical trials have begun. In such a case, generally, undifferentiated cells or differentiated cells cultured in a dedicated container alone or in the presence of a scaffold are transplanted to a target site by surgery. **SUMMARY OF THE INVENTION** Since the above-described cell transplantation method requires a surgical operation, the burden on the patient is great. Therefore, a method for directly injecting cells into a living body has been studied. In particular, injection of chondrocytes or progenitor cells thereof into joints, injection of nerve cells or progenitor cells thereof into the brain, injection of cardiomyocytes or progenitor cells thereof into the heart can be effective treatment methods. When such treatment is performed, in the conventional method, cells that have grown and adhered to the container wall are treated with trypsin or EDTA to be detached, and a predetermined amount is collected in a syringe through a process such as washing. It will be injected into the living body. However, in this method, not only the operation steps are troublesome, but also a series of operations must be performed by a skilled person in a clean environment so as not to cause contamination from the surroundings. For this reason, it is difficult to carry out cell transplantation treatment except in a well-equipped facility. [0005] An object of the present invention is to provide a medical device capable of simply carrying out cell transplantation treatment. That is, the present invention provides a medical instrument that allows anyone to easily store cells and inject them into a living body. **Means for Solving the Problems** In the present invention, the above-mentioned problems are solved by storing the proliferated cells in a container whose inner surface is formed of a material that is difficult to adhere to the cells. That is, the present invention is a cell storage container in which the inner surface of the container is formed of a material that is difficult to adhere to cells. By forming the inner surface of the container from a material that does not easily adhere to the cells, the cells do not adhere to the container wall surface during storage, and the operation of peeling the cells from the container wall surface becomes unnecessary. The phrase "cells are difficult to adhere" means that the cells do not adhere at all during cell storage or adhere only to such an extent that they can be easily detached even if they are attached. **BEST MODE FOR CARRYING OUT THE INVENTION** The cell storage container of the present invention is formed of a material whose inner surface is difficult to adhere to cells. However, if the container has a function of a syringe, it is directly injected into a living body. It is convenient because it can be done. In addition, it is preferable to form part or all of the container with a gas-permeable material because oxygen and carbon dioxide gas necessary for cell survival can be transmitted even in a sealed state, and cells can be stored for a long period of time. **EXAMPLES** Examples of materials that are difficult to adhere to cells used in the present invention include materials whose surface is formed of a hydrophilic material or a hydrophobic material and materials having a negative charge on the surface. The hydrophilic material preferably has a water contact angle of 50 degrees or less, and the hydrophobic material preferably has a water contact angle of 100 degrees or more. Examples of preferred hydrophilic materials include acrylamide polymers, methacrylamide polymers, polyacrylic acid, polymethacrylic acid, polyvinyl alcohol, polyethylene glycol, polyvinyl pyrrolidone, cellulose, dextran, hyaluronic acid, glycosaminoglycan, proteoglycan, Examples thereof include materials in which carageenan and protein are bonded to the surface of the base material by a method such as graft copolymerization or chemical reaction or coated on the surface. In addition, examples of the hydrophobic material include fluorine resins such as polytetrafluoroethylene and tetrafluoroethylene-hexafluoropropylene copolymer, and silicone resins. Examples of the material having a negative charge on the surface include materials in which polyacrylic acid, polymethacrylic acid, styrenesulfonic acid, alginic acid, heparin, heparan sulfate, chondroitin sulfate, or dermatan are bonded to the surface. Among these, a material having a carboxyl group on the surface is particularly preferable. In addition, a smooth surface of the material is preferable because

of excellent cell non-adhesiveness. In the present invention, as a method of forming part or all of the container with a gas permeable material, a method of forming part or all of the side wall of the container with a non-porous material having good gas permeability, A method of attaching a porous membrane to a part of the container so that gas exchange can be performed can be mentioned. Examples of materials having good gas permeability include silicone resin, poly-4-methylpentene 1, polyisoprene, polybutadiene, ethylene vinyl acetate copolymer, low density polyethylene, and polystyrene. These materials have relatively good gas permeability among plastic materials. However, since the gas permeability decreases as the thickness increases, it is usually preferably 200 µm or less, and preferably 100 µm or less. Particularly preferred. In the present invention, the entire container can be formed of such a material, or only a part thereof can be formed of such a material. The required gas permeability varies depending on the surface area of the container, the amount of cells filled, the type of cells and the storage conditions, but it must be sufficient for the cells filled in the container to survive. . Other examples of the gas permeable material include porous membranes. In the case of a porous membrane, the pore diameter needs to be a predetermined value or less in order to prevent the liquid inside the container from leaking. A preferable pore diameter is 1 µm or less, and it is particularly preferably 0.4 µm or less from the viewpoint of preventing liquid leakage and preventing bacteria from entering the container. Examples of the material for the porous film include polytetrafluoroethylene, tetrafluoroethylene-hexafluoropropylene, polyethylene terephthalate, and polypropylene. These porous membranes may be used on the entire side wall of the container or a part thereof. As another method of using the porous membrane, there is a method of attaching the porous membrane to the container mouth. That is, if a cap with a porous membrane attached is fitted to the container mouth, gas exchange is performed in this portion, so that other portions of the container may be gas-impermeable. As described above, if the container of the present invention has a function as a syringe, it is convenient because cells stored in the container can be directly injected into the living body. The syringe can have a structure similar to that of a general syringe that injects drugs, etc., but if it can push out the cell-containing liquid stored inside, it has a structure and principle different from those of conventional syringes. There may be. That is, a general syringe is composed of an outer cylinder, a syringe needle connecting portion formed at the distal end of the outer cylinder, and a piston. However, such a structure may be used, and the container may be formed into a bellows shape or be flexible. When extruding the internal cell-containing solution, the container may be compressed or crushed. FIGS. 1A to 1C show an embodiment in which the container of the present invention has the function of a syringe. In this embodiment, it has the same structure as a conventional syringe for drug injection, and the side surface of the outer cylinder is formed of a gas permeable material. That is, the syringe is composed of an outer cylinder 1 and a piston 2, and a gasket 3 made of an elastic material is attached to the tip of the piston 2. The outer cylinder 1 is composed of a frame 4a made of a hard material and a cylindrical gas permeable sheet 4b affixed to the inner surface of the frame 4a, and a plurality of windows forming through holes in the frame 4a. 5 is provided. Therefore, the gas permeable sheet is exposed to the outside at this window portion, and gas exchange is performed. Reference numeral 7 denotes a cap for sealing the injection needle connecting portion 6, and when the cells inside are injected into the living body, the cap is removed and the injection needle is attached. In addition, about embodiment of the window 5 in FIG. 1 shown in the present Example, although the shape of the window 5 was a rectangle and the number of the windows 5 was shown as four embodiment, this invention is restrict | limited only to this embodiment. Is not to be done. For example, even if it is another embodiment about the shape of the window 5, a dimension, and quantity, the effect of this invention is achievable. FIG. 2 is a view showing a second embodiment of the present invention. In this example, the container 8 is formed in a bellows shape, and the injection needle connecting portion 6 is provided at the tip thereof. The entire surface of the container 8 is made of a gas permeable material, and high gas permeability can be achieved. In the case of this embodiment, the structure is simpler than that of the embodiment of FIG. In addition, there is no risk of liquid leakage from the piston seal during storage. When injecting internal cells into a living body, the rear end of 8 may be directly pressed with a finger or using a jig. FIG. 3 shows a third embodiment of the present invention. In this embodiment, the cells are stored in a bag-like container 9 made of a flexible and gas-permeable material. The container 9 is provided with an injection needle connecting portion 6, and when the cells are injected into the living body, the container 9 is accommodated in the outer cylinder 1, and after the injection needle is connected to 6, the container is held by the piston 2. Press to push out the cells inside. In this example, since the cells are pushed out by the action of the piston, it is easy to operate, and the cells are stored in the container. Therefore, there is no fear of liquid leakage from the piston seal part which is a problem in the embodiment shown in FIG. Further, the outer cylinder and the piston can be used repeatedly. FIG. 4 shows a fourth embodiment of the present invention. In this embodiment, the cells are stored in the flexible bag 10 as in the third embodiment, but the bag is not provided with an injection needle connecting portion. The bag is pressed against the bag breaking means 11 provided inside 1, the bag 10 is broken, and the cell-containing liquid inside flows out into the outer cylinder and is pushed out by the piston. The bag breaking means 11 is preferably one that can easily break the bag, such as a metal blade or a needle. FIG. 5 shows a fifth embodiment of the present invention. In this embodiment, cells are stored in a bag 12 made of a flexible material. An injection needle connecting portion 6 is provided at one end of the container, and the

container 12 is squeezed by the squeezing member 13 to push cells out of the container. FIG. 6 shows an embodiment in which a gas permeable porous membrane is attached to the cap of the injection needle connecting portion to provide a gas permeable function. As can be seen from the figure, in this embodiment, the container comprises an outer cylinder 1, a piston 2, a gasket 3 and a cap 12. Further, a hydrophobic gas permeable membrane 14 is attached to the cap 7, and gas exchange is performed through the membrane 14. When the container has the function of a syringe, it is preferable to have a structure with as little cell loss as possible when extruding the cells inside. For that purpose, what is necessary is just to devise the shape of a container, the shape of a syringe connection part, etc. It is preferable to reduce the residual amount of cells since the utilization efficiency of the cells is increased. The container of the present invention is sterilized and used for cell storage. Cells are usually stored together with a medium, but in order to inject them into a living body, it is necessary to use a medium that is safe for the living body. For example, when injecting chondrocytes or progenitor cells into osteoarthritis patients, injecting nerve cells or progenitor cells into the brain of Parkinson's disease patients, and injecting cardiomyocytes into patients with heart disease, etc. When injecting cells into humans, a synthetic medium not containing biological components such as bovine serum or a medium using patient's own serum is preferable. There are no particular restrictions on the cells housed inside, but stem cells cultured by cell engineering techniques or cells obtained by differentiating stem cells into target cells are preferred. Also, preferred are genetically modified cells obtained by modifying these cells by genetic engineering techniques. Stem cells include embryonic stem cells (ES cells), embryonic germ cells (EG cells) and somatic stem cells (adult stem cells: adult stem cells; AS cells). Cell lines to be induced include bone cells, chondrocytes, muscle cells, cardiomyocytes, nerve cells, tendon cells, fat cells, pancreatic cells, hepatocytes, skin (epidermal cells / fibroblasts), blood cells, etc. I can give you. Although the container of the present invention is used for cell storage, it may be used as a cell culture container and then stored as it is. When a scaffold is required for culturing, it is preferable to use microparticles made from a material to which cells can adhere. Among such materials, those formed from a bioabsorbable material are preferable because they are absorbed into a living body and do not remain. Examples of suitable materials include polylactic acid, polyglycolic acid, lactic acid-glycolic acid copolymer, lactic acid-caprolactone copolymer, trimethylene carbonate, polydioxanone and collagen. The microparticle is preferably porous because it can adhere a large amount of cells. Cell culture can be carried out by a known method. That is, cells are separated from a living body, stem cells are selectively separated from the cells, and then added with cell growth factor or growth factor and cultured. The culture is preferably carried out in an incubator. The cultured cells are stored in the container of the present invention and stored under conditions suitable for storage until they are needed for treatment. The storage is preferably performed at a low temperature, but can be stored at room temperature or under heating for a short period of time. By injecting the cells stored in the container of the present invention into the affected part or vein, osteoarthritis, rheumatoid arthritis, pseudo-joint, progressive muscular dystrophy, myocardial infarction, stroke, Parkinson's disease, spinal cord injury Applied to the treatment of tendon injury, diabetes, liver dysfunction, digestive dysfunction, skin damage, leukemia, blood system diseases, etc. If the cells are stored using the container of the present invention, the cell detachment operation and the washing operation from the culture container, which are necessary in the conventional method, become unnecessary. Further, if the container has the function of a syringe, cells can be injected into the living body as it is, and thus regenerative medicine can be easily and safely performed in a facility that is not equipped with facilities. Furthermore, if cells are also cultured in the container of the present invention, culture, storage, and living body injection can be performed by simple operations, which is most efficient. If at least a part of the container is formed of a gas permeable material, there is an advantage that cells can be stored for a long period of time.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is an overall perspective view showing an embodiment in which a container of the present invention has a function of a syringe, FIG. 1B is a front view of the container of the present invention, and FIG. FIG. 2 is a cross-sectional view taken along line XX' in FIG. 2B. FIG. 2 is a front view showing a second embodiment of the present invention having the function of a syringe. FIG. FIG. 4 is a partial sectional front view showing a third embodiment of the present invention. FIG. 4 is a partial sectional front view showing a fourth embodiment of the present invention. FIG. 6 is a front view showing a fifth embodiment of the present invention. FIG. 6 is a front view showing an embodiment of the present invention in which a gas permeable material is provided at the injection needle connecting portion. 1. Syringe barrel Piston 3. Gasket 4a. Frame 4b. 4. Gas permeable sheet Window 6. 6. Needle connection part Cap 8. 8. Bellows container Bag-like container 110. Bag-like container 211. Bag breaking means 12. Bag-like container 313. Ironing member 14. Porous membrane