Basics of creating media

Media is used to separate purified culture of microbe from researching material and to learn its properties. Nowadays, canned dry media are used in bacteriological researches water soluble hydroscopic powder.



Important characteristic of media:

1. It should consist microorganism substances necessary for growth (organic and non organic elements)
2. It should have optimal PH reaction for growing the species of microbe
3. It should consist water, because microbes feed with diffusion and osmosis
4. Characterized with isotones
5. Be sterilized and make growth of purified microbial culture

There are two types of media by concentration **solid, semi-solid** and **liquid,**

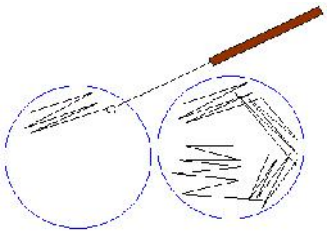
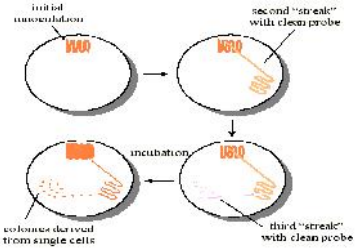


by function we can differ several types of media:

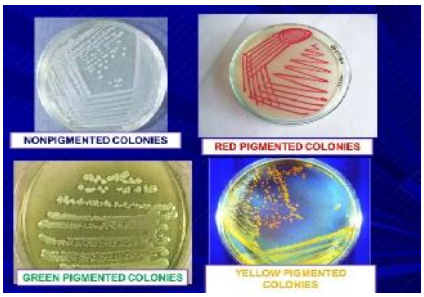
1. Essential (media which can be used for cultivating microorganisms: Brain Heart Infusion Agar, Brain Heart Infusion Broth, Nutrient Agar, Nutrient Broth)
2. Enriched media (blood and Chocolate Agar)
3. Differentiation and selective (Endo, Salmonella-Shigella, Manitol-Salt Agar)
4. Specialized (Simmonse Citrate Agar, Kligler Agar)

Solid media consists 1.5 % Agar-Agar, which characterizes its hardness. Melting temperature is 100^0C, but it gets solider at 40^0C - 45^0C.

Investigated material is planted on surface of solid media by “streak” method.

Microorganisms are grown on the surface of media and depending on species gives us different shaped size and color colonies. For cultivating of different species of micro organisms are needed different time and temperature.



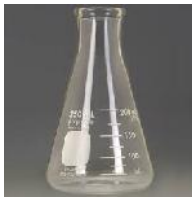
For preparing any type of media in microbiological laboratory is separated a special area which is called “clear area”. This territory is safe from microbial contamination. This ”clear area” stays untouched from investigated materials. Laminar box is must have thing in this area, which is used only for pouring the media.

Also there are laboratory tables and cardboards for keeping laboratory glasses, purified water container, sink, microwave oven, stove or electro heater.

Person preparing the media should be dressed in lab coat and gloves.

It is necessary to have prepared in advance sterile closed gapped glass dishes.

Glass fireproofs Flask, glass stick, electric balance and sterile plastic or glass Petri dishes.




Get to know media creation instructions which are written on the appropriate box.

According to instruction: we take one liter of desterilized water (which can be found in every bacteriological laboratory. Purified water container is seated besides wet place) and pour it in the glass fireproof Flask. (The glass fireproof Flask is graduated, gaps tells about its capacity).

Measurement plate is placed on electro balance to find out its weight. According to results from media box to measure plate we place the powder 52 grams with special spoon. Measured powder is carefully placed in glass fireproof Flask (where we have already placed distil water), place on the stove or electro heater and by glass stick stir until dry powder dissolves. The resulted liquid should be boiled.

We take paper indicator (this indicators are placed in little plastic box) used for PH reaction.


We grab this paper indicator on the one end and place it half into the prepared liquid.

The PH of media is checked by comparing of colored paper indicator to the colors mentioned on the box. Colors mentioned on the box matches numbers. (it is different for different microorganism species). If the PH of media is not satisfied, according to alkali or acid reaction we want to get - we add NAOH or HCl. The optimal PH for most bacterial species is 7,2±0,2. For this purpose also can be used PH meter.

Prepered media we poure in the sterail gapped glass boteles, close the lid and cover with foil paper. We wash glass fireproof Flask and put in its place.

In the gapped glass bottles with capacity 250ml we put 150ml media and in the 500ml bottles - 300ml. Thus, we avoid lid wetting during autoclave and melting process.

On glass bottles with media we put paper ribbon and label with marker – nomination, percentage, the date of preparing. After we put the bottles in the autoclave.

Sterilization process is going 15mn on 121^{0}C. After time out and autoclave become cooler we take out media, wait until it's become solid and keep in cool place (refrigerator) + 4-5^{0}C.

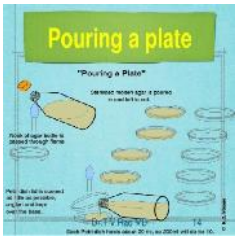
Before using we melt the media - put glass bottles in the microwave oven or in the watery dishes and place on the stove or electro heater. Media should be poured in melt condition (not very hot) on sterile plastic or glass Petri dishes in Laminar box.

Before we start work in Laminar box we switch it on. The front glass put down and switch on UV light use button on the control panel of box.

After 15-20mn we switch of the UV light by the same button and by help of another button switch on the air source and light. Thus, the box is ready for use.

Before working we take a place in front of box, put sterile plastic or glass Petri dishes and melted media in the box, carefully unpack bottle from foil paper and cover.

Take bottle in left hand, with right hand take up half the Petri dishes cover and pour 30ml of media, after quickly put down the cover in initial condition. We do the same until bottle is empty.



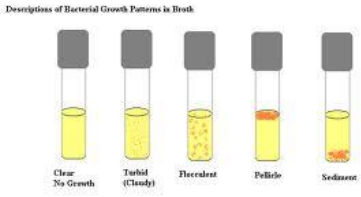
Media on Petri dishes become solid and should be kept with cover down to avoid accommodation of big amount of condensate on the media surface. We label Petri dishes by marker – type of media and date.

Petri dishes with media we keep in the refrigerator on + 4-5^{0}C. We take out the bottle from the box, wash and put it in the cupboard were we keep non sterile dishes.

Pour of media is possible near the flame, if there is burner in the lab.

In these case we put sterile Petri dishes near the berner and make all above described procedure near the burner.

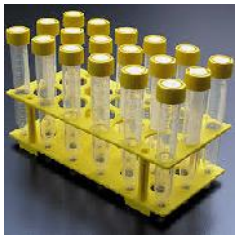
Liquid media or "broth" dose not consists Agar-Agar. For cultivating of different species of microorganisms are needed different time and temperature and depending on species bacteria gives us different type of growth.



Investigated material is possible to culture in the media by help of sterile plastic or metallic loop or sterile plastic or glass pipet.

Procedures for preparing liquid media and Laminar box in work condition is the same as it mentioned above. There are some differences after media is ready and we want to pour it in sterile plastic or glass tubes.

Before we start pour “broth” we should take and place in Laminar box: prepared sterile “broth” in bottle (carefully unpack it from foil paper and cover); stand with sterile plastic or glass tubes and pipette; pump.



Unpack pipette, fix the pump on it and hold in right hand, bottle with “broth” in left hand.

By free fingers of right hand remove cover from bottle, carefully put pipette into the “broth” and take 4.5ml of liquid, put cover on its initial place, pot bottle on the table and with the same hand pick up from the

stand tube, by free fingers of right hand remove cover from tube and pour media in the tube. Repeat these procedures until bottle is empty. We label tubes by marker – type of media and date.

Pour of media is possible near the flame, if there is burner in the lab.

In these case we put sterile tubes near the berner and make all above described procedure near the burner.