INTERNATIONAL STANDARD

ISO 8573-7

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Compressed air —

Part 7: Test method for viable microbiological contaminant content

Air comprimé —

Partie 7: Méthode d'essai pour la détermination de la teneur en polluants microbiologiques viables

Reference number
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Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 8573-7 was prepared by Technical Committee ISO/TC 118, *Compressors, pneumatic tools and pneumatic machines*, Subcommittee SC 4, *Quality of compressed air*.

ISO 8573 consists of the following parts, under the general title *Compressed air*:

— *Part 1: Contaminants and purity classes*

— *Part 2: Test methods for aerosol oil content*

— *Part 3: Test methods for measurement of humidity*

— *Part 4: Test methods for solid particle content*

— *Part 5: Test methods for oil vapour and organic solvent content*

— *Part 6: Test methods for gaseous contaminant content*

— *Part 7: Test method for viable microbiological contaminant content*

— *Part 8: Test methods for solid particle content by mass concentration*

— *Part 9: Test methods for liquid water content*

Compressed air —

Part 7:
Test method for viable microbiological contaminant content

1 Scope

This part of ISO 8573 specifies a test method for distinguishing viable, colony-forming, microbiological organisms (e.g. yeast, bacteria, endotoxins) from other solid particles which may be present in compressed air. One of a series of standards aimed at harmonizing air contamination measurements, it provides a means of sampling, incubating and determining the number of microbiological particles. The test method is suitable for determining purity classes in accordance with ISO 8573-1, and is intended to be used in conjunction with ISO 8573-4 when there is need to identify solid particles that are also viable, colony-forming units.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8573-1, *Compressed air — Part 1: Contaminants and purity classes*

ISO 8573-4, *Compressed air — Part 4: Test methods for solid particle content*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
microbiological organisms
particles characterized by their ability to form viable colonies

NOTE These can be identified as bacteria, yeast or fungi.

3.2
number of viable micro-organisms
number of micro-organisms having a potential for metabolic activity

3.3
culturable number
number of micro-organisms, single cells or aggregates able to form colonies on a solid nutrient medium

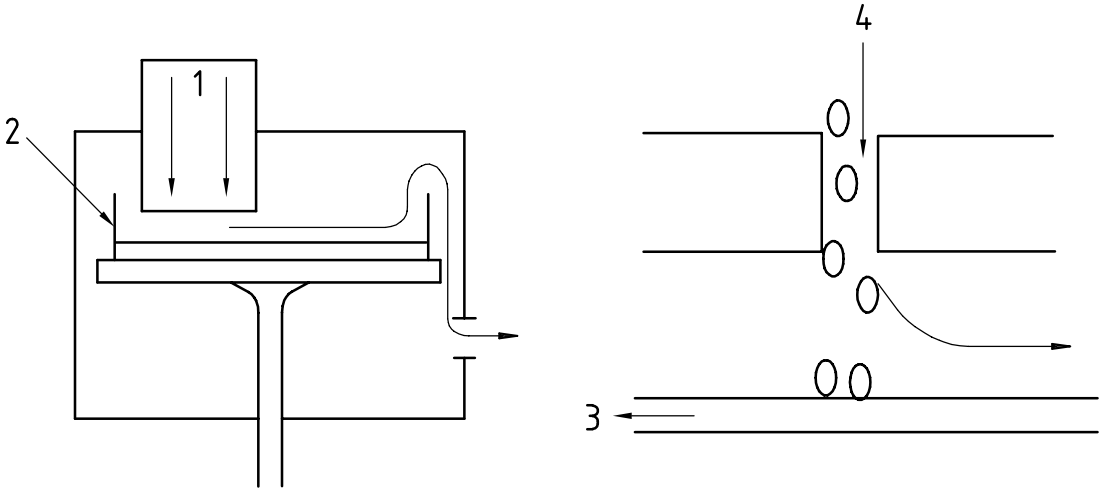
3.4
colony-forming unit
CFU
unit by which the culturable number is expressed

4 Method for verifying presence of viable micro-organisms by partial flow sampling

The method for verifying the presence of viable micro-organisms is to expose an agar nutrient to the compressed air sample. A quantitative assessment may be made by the method given in Annex B. See Annex D for details on the preparation of an agar plate with a culturable media.

For partial-flow sampling, a slit-sampler, a type of impaction air tester (see Figure 1), shall be used together with the method given in ISO 8573-4. Isokinetic sampling of the air shall be carried out and reduced until it is within the range of the sampler as identified by the manufacturer. Pressure reduction to atmospheric conditions and flow measurements shall be performed in order to establish compatibility with the manufacturer's recommendations or in accordance with ISO 8573-4. Where the flow is known, the time for the exposure of the agar media to the compressed air sample shall be recorded.

To assist in discriminating non-microbiological from microbiological particles, measurements shall be taken within 4 h.



Key

1 air intake
2 rotating Petri dish with agar
3 air outlet
4 air

Figure 1 — Slit-sampler

It is necessary to eliminate, as far as possible, the influence of liquids on particle size and number so that a correct reading can be obtained. The influence of water shall not be reduced by heating or drying of air, where this might otherwise have been appropriate, in order to avoid influencing the viability of microbiological organisms.

The influence of liquids other than water shall be given due consideration.

5 Operating conditions

Actual operating conditions shall be described in the test report (see Annex A).

6 Determination of viable, colony-forming organisms

After incubation of the sample on the agar nutrient (see B.3), the surface shall be visually examined to confirm the presence of viable, colony-forming micro-organisms.

7 Test report statement

A statement shall be made in the test report, supplementary to the statement in accordance with ISO 8573-4 for solid particles, providing confirmation that there are viable, colony-forming microbiological particles among the solid particles.

This phrase “Declared sterility of the compressed air in accordance with ISO 8573-1”, shall be followed by

— “Sterile” or “non-sterile”,

— date of sampling,

— date of measurements, and

— location.

Annex A presents a sample test report.

Annex A
(informative)

Determination of viable microbiological particle content in compressed air — Sample test report

Once the solid particle content in accordance with ISO 8573-4 has been established, a tabulated test report (see Figure A.1) is used to identify those particles present as viable microbiological CFUs in a sample of air taken from the compressed air system under investigation.

NOTE For information on agar media, see B.3

[Value of actual, average measured value (see Annex B) **for …**	
Microbiological organism	**CFU/m^3** given at reference conditions[a]
Bacteria	100
Yeast	14
Fungi	No indication
Endoro-bacteria	50
Pressure to which the measurement refers	… MPA [= bar (e)]
[Statement regarding the applicable uncertainty … (see Clause 7)**]**	
Date of calibration record	yyyy/mm/dd

[a] Reference conditions:

Temperature 20 °C;

Pressure 0,1 MPa (= 1 bar).

Relative humidity does not affect volume in this application.

Figure A.1 — Sample test report

Annex B
(normative)

Quantitative sampling method

B.1 Sampling with slit-sampler (see Figure 1)

B.1.1 Principle

The principle of capturing micro-organisms with a slit-sampler (impaction air tester) is both simple and reliable. Air from a compressed air installation is channelled through a specially designed connecting link and accelerated through a narrow slit towards a moist agar surface. The micro-organisms, due to their weight, are flung into the agar surface, whereas the air molecules are deflected. Suitably incubated, they multiply into colonies, which are counted on the assumption that one micro-organism gives rise to one colony.

The slit-sampler can be used for bacteria, yeast or fungi and, with special methods, for viruses and bacteriophages. As a large agar surface (e.g. 140 mm Petri dish) rotates under a radially positioned slit (0,5 mm), a large number of colonies, i.e. organisms, can be counted.

B.1.2 Aseptic techniques

The sampling methodology is covered by the adoption of aseptic techniques. The use of a disinfecting agent such as 70 % ethanol is recommended. In periods when the slit-sampler is not in use (stored) precautions shall be taken to avoid the growth of micro-organisms in the equipment. All operations in which the test equipment is to be opened should be carried out with the minimum of delay in order to avoid possible ingress of contaminants from the local environment. Precautions should also be taken against the effects of draughts.

B.2 Sampling procedure

The following procedure shall be used for sampling.

a) Sterilize all sampling equipment by disinfecting the equipment, including tubes and hoses, with a suitable cleaning agent immediately before use.

b) Allow a test sample to pass through the sampling equipment and associated tubes and hoses without the Petri dish and agar. This is done to allow evaporation of the disinfecting agent and to adjust the slit-sampler.

c) Perform a blind test before, and after, the actual measurement by carrying out steps d) to f) without starting the slit-sampler. The dishes used shall not subsequently show growth.

d) Take a 14 cm Petri dish with agar. The Petri dish shall have a label fixed to the bottom with traceability information (date, time of start, test site address, code, etc). Indicate the starting position and the direction of rotation.

e) Ensure that the air inlet and level indicator of the slit-sampler are turned up. Lift the lid of the slit-sampler and ensure that the plate holder is placed correctly in relation to the micro-switch. Wipe the internal sides of the slit-sampler with a disinfecting pad.

f) Insert the Petri dish in the slit-sampler, which should be exposed so that the radial line is situated directly under the air inlet slit. Remove the lid and store it in a sterile plastic bag.

g) Quickly replace the lid of the slit-sampler immediately after removing the Petri dish lid.

h) Loosen the level indicator and lower it carefully onto the surface of the agar. Lower the air inlet so the indicator arrow points directly at the inferior edge of the groove track. Raise the level indicator again to its upper position and fasten it.

i) Start the automatic sampling by pressing the start button. Make a note of the starting time, sampling time, test premises and other conditions or observations that could influence the measurements.

j) When the pilot lamp is switched off, the sampling is finished. With the start/stop button in the off-position, raise the air inlet.

k) Loosen the lid of the slit-sampler and carefully lift it, at the same time removing the lid of the Petri dish from the sterile plastic bag and replacing it on the Petri dish. Due care and attention shall be observed in this process so as not to disturb the agar and sample.

l) Remove the Petri dish from the sampling equipment and replace the lid of the slit-sampler. Seal the Petri dish with tape and replace it in the sterile bag, then seal the bag with tape.

m) Petri dishes are incubated at a convenient temperature and read after a suitable time. See B.3. At the centre and outer edge, the agar surface shall be free from colonies.

NOTE The start/end line could contain “extra” colonies.

n) Move the activation arm of the dish holder past the micro-switch to a new start position.

o) Wipe the inside of the slit-sampler with a disinfecting pad. Replace the lid on the slit-sampler.

p) Restart this procedure from the beginning when performing a new sampling.

Using the same means of transport, “geographically” trace a Petri dish the entire distance from the manufacturer who filled the Petri dishes with agar, to the place of sampling and the laboratory, in order that it can be inspected for unintended after-contamination. The dish shall not subsequently show growth.

B.3 Incubation of viable organism contaminant

In general, the most appropriate temperature during incubation is that near to the habitat in which the micro-organisms were present before sampling. Mesophyllic bacteria or fungi should be cultivated at temperatures from 20 °C to 30 °C. For specific thermo-tolerant bacteria other temperatures may be requested. Incubation periods of up to fourteen days are normal for fungi, while those for mesophyllic bacteria normally vary from two to fourteen days. Other incubation temperatures may be considered.

Selective media (agars) may be used for isolation of, for example, gram-negative enterobacteria; the counting shall take place within a given time period (e.g. 24 h).

B.4 Measurement of CFUs

Non-selective media may be examined and the growth counted as early as 24 h after the beginning of incubation and then recounted every 24 h for ten to fourteen days. Regular observations shall be made during the incubation period to count and record colonies as they emerge and to prevent loss of counting accuracy by overgrowth of colonies.

Annex C
(informative)

Sampling endotoxins

C.1 General

The sampling of endotoxins in compressed air is a difficult process requiring the use of virgin plastic tubes and glass bottles as well as personnel experienced in the required techniques. However, it is possible to identify the presence of endotoxins in compressed air by measuring the quantity of gram-negative enterobacteria organisms in compressed air condensate.

Nevertheless, a supplementary measurement of the content of bacteria, fungi or yeast in the condensate should be carried out.

C.2 Sampling procedure

WARNING — Just a few nanograms of endotoxins (waste-products from gram-negative bacteria) in the compressed air could cause illness.

The following procedure shall be used for measuring gram-negative enterobacteria in condensate. Sterile work practices shall be adopted at all times. A dip-stick with a suitable agar medium shall be used. The test-point shall be a convenient point in the compressed air system under investigation where condensate can be collected.

a) Disinfect the test point with 70 % ethanol immediately before sampling.

b) Remove the vial lid with the attached slide coated with the agar media.

c) Take a sample of condensate from the test-point directly into the sterile vial.

d) Dip the lid with the attached slide in the sample for a period of 10 s. It is important that both agar-surfaces come into intimate contact with the specimen.

e) Slowly withdraw the slide from the sample during approximately 3 s.

f) Drain off the vial.

g) After inoculation, carefully replace the slide in the vial. At this stage the vial with its slide can be stored or transported for hours without affecting the result. Never allow the vial containing the sample slide to freeze.

h) Incubate the slides at + 27 °C for up to fourteen days. If the organisms grow very slowly, the incubation period can be extended to up to a month.

i) After incubation, very carefully remove the slide from the vial. Examine growth and colour-reactions according to the manufacturer's instructions.

The acceptable level for bacteria, yeast and fungi is 10 000 CFU/ml condensate. If one gram-negative bacterium is found in the condensate, then endotoxins are present in the compressed air, and the wet part of the installation shall be cleaned and disinfected.

Annex D
(informative)

Preparation of Petri dish with culturable media

The following procedure is valid for both culturable media, plate count agar and Saboroud with 4 % dextrose.

a) Weigh out the culturable media in the quantity specified by the manufacturer and dissolve it in water.

b) Autoclave the culturable media at 121 °C for 15 min.

c) After cooling to about + 50 °C, measure the pH and, if necessary, adjust it to the stated pH using either hydrochloric acid or sodium hydroxide.

d) Using sterile, 14 cm plastic Petri dishes, pour 65 ml of the culturable media into each dish.

e) When the culturable media is cool and stiff, pack each Petri dish in two sterile plastic bags:

   1) close the first bag with a simple double fold-over seal;

   2) seal the second bag positively with a welded edge.

f) Label the dish with information about date, content and batch number.

Bibliography

[1] ISO 4833:2003, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms — Colony-count technique at 30 °C*

[2] ISO 7218, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*

[3] ISO 7954, *Microbiology — General guidance for enumeration of yeasts and moulds — Colony counting technique at 25 °C*

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