INTERNATIONAL STANDARD

ISO 9252

First edition
1989-11-15

Synthetic rubber latex — Microbiological examination

Latex de caoutchouc synthétique — Examen microbiologique

Reference number
ISO 9252:1989(E)

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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 9252 was prepared by Technical Committee ISO/TC 45, *Rubber and rubber products*.

Annex A of this International Standard is for information only.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland

Printed in Switzerland

Introduction

Synthetic latices are susceptible to post-production contamination with micro-organisms during storage and shipment. Unless precautions are taken such as maintenance of a high pH, the addition of biocide and inspection and cleaning of tanks, these organisms may proliferate, ultimately producing unpleasant odours and changes in the chemical and physical properties of the latex. It is highly desirable to be able to detect the presence of significant micro-organisms before such changes develop.

The method described can be carried out by any reasonably competent general testing laboratory, provided the operator has been trained in basic microbiological techniques. Alternatively, the method would be routine for a specialist microbiological laboratory.

INTERNATIONAL STANDARD ISO 9252:1989(E)

Synthetic rubber latex — Microbiological examination

1 Scope

This International Standard specifies a method for the microbiological examination of synthetic rubber latices for the presence and approximate concentration of viable aerobic and facultative anaerobic micro-organisms.

Identification of the micro-organisms is outside the scope of this International Standard, though a skilled microbiologist will be able to derive some information on them from recovered cultures.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 123:1985, *Rubber latex — Sampling.*

ISO 976:1986, *Rubber latices — Determination of pH.*

ISO 4833:1978, *Microbiology — General guidance for enumeration of micro-organisms — Colony count technique at 30 °C.*

ISO 6887:1983, *Microbiology — General guidance for the preparation of dilutions for microbiological examination.*

ISO 7218:1985, *Microbiology — General guidance for microbiological examinations.*

3 Definition

For the purposes of this International Standard, the following definition applies.

colony: A group of microbial cells derived, ideally, by the multiplication of a single organism.

4 Principle

A nutrient medium is selected on the basis of the pH of the latex. The test portion of the latex is serially diluted and aliquots of each dilution are spread over the surface of a solid medium in a Petri dish, followed by incubation at 30 °C for 3 days. The number of colonies of micro-organisms that develop are counted, multiplied by the dilution factor and recorded as colony-forming units per cubic centimetre of the original sample.

5 Reagents

Unless otherwise stated, use only distilled water or water of equivalent purity.

NOTE 1 Distilled water is not necessarily sterile.

5.1 Ethanol, 70 % (*m*/*m*) aqueous solution (commonly called industrial grade).

5.2 Dilution fluids.

5.2.1 Sodium chloride/peptone solution (see A.2.1).

5.2.2 Quarter-strength Ringer solution (see A.2.2).

5.3 Media.

5.3.1 Plate count agar (see A.4.1).

5.3.2 Sabouraud dextrose agar (see A.4.2).

5.3.3 Beef extract agar (see A.4.3).

6 Apparatus

6.1 Steam autoclave, capable of maintaining a temperature of 121 °C ± 1 °C, as described in ISO 7218.

6.2 Incubator, capable of maintaining a temperature of 30 °C ± 1 °C.

6.3 Sterile pipettes, of capacity 1 cm^3 and 0,1 cm^3. These may be pre-sterilized disposable pipettes or, if glass pipettes are used, they shall be clean and sterilized before use.

6.4 Petri dishes.

These may be obtained ready-prepared from laboratory supply houses. They may also be prepared and sterilized in the testing laboratory (see informative annex A), provided that it is equipped to do so.

6.5 Illuminated colony-counting equipment, as described in ISO 4833.

7 Sampling

Sampling shall be carried out as described in ISO 123, using pre-sterilized, disposable equipment or equipment sterilized by being kept at 121 °C ± 1 °C for not less than 20 min in an autoclave (6.1).

It may be necessary to take samples of material from the surface of the latex as well as from the body, since the different conditions prevailing at the surface sometimes enhance the growth of micro-organisms.

8 Selection of media

8.1 General

If the pH of the latex is unknown, determine it on a separate sample in accordance with ISO 976, but do not use this sample for subsequent microbial analysis.

Synthetic latices differ widely in pH. The pH of the latex materially affects the types of organism that will grow in it. Thus it may be necessary to use more than one type of medium as indicated below. Experience will indicate the preferred medium for a given latex. Incubations shall be carried out at 30 °C ± 1 °C for the times indicated below.

Details of the preparation of media and plates are given in informative annex A.

8.2 Synthetic latices with pH greater than 7

Use plate count agar (5.3.1) and incubate for 3 d ± 2 h, or

use beef extract agar (5.3.3) and incubate for 5 d ± 2 h.

8.3 Synthetic latices with pH less than 7

Use plate count agar (5.3.1) and incubate for 3 d ± 2 h, or

use sabouraud dextrose agar (5.3.2) and incubate for 5 d ± 2 h, or

use beef extract agar (5.3.3) and incubate for 5 d ± 2 h.

9 Procedure

These operations shall not be carried out in direct sunlight.

9.1 Test sample

Thoroughly mix the sample, taking sterile precautions.

9.2 Preparation of dilutions

Prepare dilutions in accordance with ISO 6887 (see also annex A), using sodium chloride/peptone solution (5.2.1) or quarter-strength Ringer solution (5.2.2).

9.3 Inoculation of Petri dishes

Two Petri dishes (6.4) shall be prepared from each dilution.

Take a fresh sterile 0,1 cm^3 pipette (6.3) and, using the mixing technique specified in 9.2, inoculate 0,1 cm^3 on to a Petri dish containing the selected medium. Take a sterile L-shaped glass rod and spread the inoculum quickly over the surface of the Petri dish. Replace the lid and wait until the inoculum is adsorbed. Sterilization of the glass rod can be achieved by dipping it into ethanol (5.1) and burning off the ethanol.

Repeat the process, using the same dilution, to prepare a second Petri dish.

Using a fresh sterile pipette for each dilution, inoculate further pairs of Petri dishes in the same manner with the other dilutions in order of decreasing dilution.

9.4 Blank

Prepare two blanks by using 0,1 cm^3 of sterile water in place of the latex in 9.3.

9.5 Incubation of Petri dishes

Incubate the dishes in an inverted position. Do not stack more than six high. Separate stacks of dishes from one another and from the walls of the

incubator. Incubate at 30 °C ± 1 °C for the time specified in 8.2 or 8.3 as appropriate.

9.6 Counting of colonies

9.6.1 Count the colonies within 4 h of the incubation period. If this is not practical, the dishes may be stored for up to a maximum of 24 h at a temperature not exceeding 4 °C. Carry out the counting in accordance with ISO 4833.

9.6.2 Multiply the number of colonies by the dilution factor and then by 10 (to allow for the amount of the inoculum) to obtain the count.

9.6.3 If the average count of the blanks after incubation exceeds two, repeat the whole series, paying particular attention to cleanliness, sterilization and manipulative techniques.

9.7 Disposal of Petri dishes and used apparatus

All used apparatus and incubated culture media shall be sterilized or decontaminated. Glass apparatus, pipettes, etc., shall be placed in disinfectant solution (e.g. sodium hypochlorite) before washing and sterilization. Petri dishes shall be sterilized for a minimum of 20 min at 121 °C ± 1°C before disposal of the medium.

9.8 Significance of count

Because of variations between different strains of micro-organism and between different latices, it is impossible to give firm limits for degrees of contamination. However, as a rough guide it can be reported that

a) a count of < 30 shows no significant contamination by micro-organisms;

b) a count of 10^3 shows slight contamination;

c) a count of 10^4 shows contamination;

d) a count of $> 10^5$ shows heavy contamination.

10 Expression of results

The results shall be expressed as the average count per cubic centimetre of original latex, rounded to two significant figures.

Optionally, further comments may be added: for example, that the contamination appears to be a uniform strain, or a mixed culture.

11 Precision

Because of the wide range of results that may be obtained, e.g. from 10 to 10^7, and because of the inherent variability of microbiological testing, results differing by a factor of up to two are not normally considered significantly different.

12 Test report

The test report shall include the following information:

a) a reference to this International Standard;

b) all information necessary to identify the test sample;

c) the date of the test;

d) the dilution fluid used;

e) the medium or media used, with the time and temperature of incubation;

f) the count per cubic centimetre of original latex for each medium used;

g) any unusual features noted during the examination, e.g. that there appeared to be a uniform strain of micro-organisms or a mixed culture;

h) any operation not specified in this International Standard.

Annex A
(informative)

Preparation of media and plates

NOTE 2 Where facilities permit, media and plates may be prepared as described in this annex or in accordance with ISO 6887.

A.1 General

A.1.1 Materials and reagents

Use water distilled from glass apparatus or water otherwise produced, of at least equal purity.

Use granulated agar wherever agar is specified.

All reagents shall be of analytical quality where obtainable or otherwise of the highest possible quality.

Commercial brands of pre-mixed dehydrated media may be used where experience has indicated that on reconstituting they give comparable results. They shall be made up and sterilized in accordance with the maker's instructions; otherwise the procedure in A.1.2 shall be followed.

A.1.2 Sterilization of media

Sterilize media in an autoclave. Take care to ensure sterilization without overheating. The conditions necessary to achieve uniform heat treatment are as follows:

a) complete exhaustion of air from the chamber;

b) control of the process by temperature;

c) rapid heating of the chamber;

d) the employment of standard loads.

A.1.3 Temperature measurement

An accurate temperature-sensing device shall be fitted to the autoclave in a suitable thermometer pocket, sited so that the sensor lies in the path of the steam leading to the vent. This serves to indicate when the chamber is virtually free from air; it also affords a means of checking the holding temperature, provided that a trickle of steam is allowed to bleed past the sensor during holding. For this purpose, a second air vent with an adjustable valve is necessary.

A.1.4 Removal of air

To ensure complete removal of air from the chamber, the vent shall not be closed until the temperature of the exhaust steam shows that it is air-free. Owing to changes in barometric pressure, this temperature may vary by a degree or two from 121 °C, but this difficulty is overcome by closing the vent only when the increase in thermometer reading does not exceed 0,5 °C over a period of 2 min to 3 min.

A.1.5 Holding time and temperature

Sterilize at 121 °C ± 1 °C for not less than 20 min. Avoid excessive loading of the autoclave.

A.2 Dilution fluid

A.2.1 Sodium chloride/peptone solution

Composition

peptone	1,0 g
sodium chloride	8,5 g
water	1 dm^3

The peptone and sodium chloride are dissolved in the water, by heating if necessary. The pH is adjusted so that, after sterilization, it is 7,0 at 25 °C.

A.2.2 Quarter-strength Ringer solution

Composition

sodium chloride	9,00 g
potassium chloride	0,42 g
anhydrous calcium chloride	0,24 g
sodium hydrogen carbonate	0,20 g
water to make	1 dm^3

Add one part of the above solution to three parts of water to make a quarter-strength solution. Dispense this into 150 mm × ø 16 mm test tubes fitted with suitable closures, or other suitable containers, and sterilize by heating in an autoclave for 20 min ± 1 min at 121 °C ± 1 °C. With this

method, it will be necessary to dispense more than the 9 cm^3 of quarter-strength Ringer solution required (see clause A.3) into the tubes to allow for losses due to evaporation during sterilization. Alternatively, sterile tubes may be filled under aseptic conditions.

Ringer solution tablets may also be used. One tablet is dissolved in 500 cm^3 of water, and 150 mm × ø 16 mm test tubes are filled and sterilized as above.

A.3 Preparation of dilutions

Prepare dilutions just prior to the analysis. Use them for inoculating media within 30 min of preparation.

It is essential that the test sample is uniform. Remove 1 cm^3 with a sterile pipette (6.3) and add this test portion to 9 cm^3 of diluent (A.2.1 or A.2.2), avoiding contact between the pipette and the diluent. Mix this primary dilution (10^{-1}) carefully by aspirating 10 times with a clean, sterile pipette.

Further dilutions (10^{-2}, 10^{-3}, 10^{-4}, etc.) are prepared by repeating the above process.

A.4 Composition of media

A.4.1 Plate count agar
(tryptone-dextrose-yeast agar)

Composition

tryptone	5,0 g
yeast extract powder	2,5 g
dextrose	1,0 g
agar	9,0 g
water to make	1 dm^3

pH 7,0 (approx.)

A.4.2 Sabouraud dextrose agar

Composition

mycological peptone	10 g
dextrose	40 g
agar	15 g
water to make	1 dm^3

pH 5,6 (approx.)

A.4.3 Beef extract agar

Composition

beef extract	3,0 g
peptone from casein (free of fermentable carbohydrates)	5,0 g
sodium chloride	5,0 g
agar	10,0 g
water to make	1 dm^3

pH 7,3 ± 0,2

UDC 678.031:579

Descriptors: rubber, synthetic rubber, latex, tests, microbiological analysis, determination, micro-organisms.

Price based on 5 pages