INTERNATIONAL STANDARD

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Synthetic rubber latex — Microbiological examination

Latex de caoutchouc synthétique — Examen microbiologique

Reference number
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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.

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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.

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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.

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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

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