
**Synthetic rubber latex — Examination
for microorganisms**

Latex de caoutchouc synthétique — Examen des micro-organismes

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Contents

	Page
Foreword.....	iv
Introduction.....	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	1
5 Reagent	2
6 Apparatus	2
7 Sampling	2
8 Procedure	2
9 Examination	3
9.1 Examination of the microbial count (CFU/ml).....	3
9.2 Interpretation.....	3
10 Disposal of the used slides and equipment	3
11 Test report	3
Annex A (informative) Example of a model chart	4
Annex B (informative) Interpretation of the microbial count	5

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html

This document was prepared by Technical Committee ISO/TC 45, *Rubber and rubber products*, Subcommittee SC 3, *Raw materials (including latex) for use in the rubber industry*.

Introduction

Synthetic latices are susceptible to post-production contamination with microorganisms 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 microorganisms before such changes develop.

This document replaces ISO 9252:1989, *Synthetic rubber latex – Microbiological examination*, which was withdrawn due to its obsolescence and complicated testing procedures. This document provides a far more simplified method for the purpose of microbiological examination, employing a ready-made medium slide as reagent.

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Synthetic rubber latex — Examination for microorganisms

WARNING 1 — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

WARNING 2 — Certain procedures specified in this document might involve the use or generation of substances, or the generation of waste, that could constitute a local environmental hazard. Reference should be made to appropriate documentation on safe handling and disposal after use.

1 Scope

This document specifies the method to examine the presence and approximate count of viable aerobic and facultative anaerobic microorganisms in synthetic rubber latices.

Identification of the microorganisms is outside the scope of this document.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 123, *Rubber latex — Sampling*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>

3.1

colony

group of microbial cells derived, ideally, by the multiplication of a single organism

4 Principle

The slide with the medium surfaces is dipped in the test portion of the latex. The microorganisms on both sides of the slide are incubated for a certain amount of time at a regulated temperature.

After incubation, the approximate number of microorganisms is determined by comparing the density of the growth of colony on the slide with the model chart.

The approximate count is expressed in colony forming unit per millilitre (CFU/ml).

5 Reagent

During the analysis, use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

5.1 Medium slide¹⁾, designed to examine the count of viable aerobic and facultative anaerobic microorganisms, usually stored in a tube.

NOTE Typical formulation of the medium includes

- a) TTC (2,3,5-Triphenyl 2H-tetrazolium chloride),
- b) agar, and
- c) tryptone and/or peptone.

5.2 1 % (mass fraction) sodium hypochlorite solution, as disinfectant.

6 Apparatus

Standard laboratory equipment and the following.

6.1 Incubator, capable of maintaining a temperature of $28\text{ °C} \pm 1\text{ °C}$ or $36\text{ °C} \pm 1\text{ °C}$.

6.2 Autoclave, capable of maintaining a temperature of $121\text{ °C} \pm 1\text{ °C}$, as described in ISO 7218.

6.3 Absorbent paper.

7 Sampling

Sampling shall be carried out as described in ISO 123, using disposable pre-sterilized equipment or equipment sterilized in accordance with ISO 7218.

8 Procedure

Unless otherwise instructed by the manufacturers, a typical procedure is described hereafter. Operations shall be carried out in accordance with the instruction by the manufacturer of the medium slide.

- a) Agitate the sample thoroughly.
- b) Unscrew the tube and withdraw the slide without touching the medium surfaces.
- c) Inspect both sides of the slide to determine if there are any defects on the growth media such as discoloration or detachment from the plastic slide.
- d) Dip the slide in the sample for approximately 5 s to have both sides of the slide completely wet. If the viscosity of the sample is high, dilute the sample with sterile water and record the dilution factor, *F*.
- e) Drain off excess sample and blot the last drops from the lower end of the slide on absorbent paper (6.3).
- f) Screw the medium slide tightly back into the tube.

1) SAN-AI BIOCHECKER® TTC supplied by SAN-AI OIL CO., LTD. (www.san-ai-oil.co.jp/) and Easicult® TTC supplied by Orion Diagnostica Oy (www.oriondiagnostica.com) are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

- g) To prepare a blank, withdraw another medium slide from a tube and expose it to the air in the test atmosphere for approximately 30 s. Screw the slide tightly back into the tube.

NOTE The exposure time to the air is determined based on the time required for the procedure from b) to f).

- h) Incubate the sample tube and the blank tube together at $28\text{ °C} \pm 2\text{ °C}$ for $48\text{ h} \pm 2\text{ h}$, or at $36\text{ °C} \pm 2\text{ °C}$ for $24\text{ h} \pm 2\text{ h}$.

9 Examination

9.1 Examination of the microbial count (CFU/ml)

Within 4 h after the incubation, remove the sample slide and the blank slide from each tube carefully and compare the density of colonies with the model chart. Comparison for both sides of the slide shall be made and the approximate microbial count (CFU/ml) shall be given in powers of 10 as the count per cubic centimetre of original latex. An example of the chart is given in [Annex A](#). For actual use, make sure to refer to the chart which is provided by the medium slide manufacturer.

Compare the counts on both sides ($n = 2$) and report the higher one. The result shall be given ranging from less than 10^3 , 10^3 , 10^4 , ... to 10^7 .

When the sample is diluted, the dilution factor, F , shall be taken into account in the evaluation. The actual result shall be calculated by multiplying the microbial count (CFU/ml) of the diluted sample by F .

If the higher count of the blank exceeds 10^3 , repeat the whole procedure carefully.

9.2 Interpretation

For interpretation of the microbial count, see [Annex B](#). Further comments may be added.

10 Disposal of the used slides and equipment

Used slides and tubes shall be either burnt or disposed of after being sterilized/decontaminated by immersing in 1 % sodium hypochlorite solution ([5.2](#)) overnight.

All used equipment and glass equipment shall be placed in 1 % sodium hypochlorite solution ([5.2](#)) over 2 h and either washed or sterilized by autoclaving at $121\text{ °C} \pm 1\text{ °C}$ for more than 20 min.

11 Test report

The test report shall include the following information:

- a) a reference to this document, i.e. ISO 20851;
- b) all information necessary to identify the test sample;
- c) the dilution factor, F (when applicable);
- d) the time and the temperature of incubation;
- e) the microbial count (CFU/ml) of the test sample;
- f) any operation not specified in this document;
- i) the date of the test.