© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. 014/009/26-295, valid until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

ISBN 978-0-626-39070-9

SANS 21148:2020
Edition 2

ISO 21148:2017
Edition 2

SOUTH AFRICAN NATIONAL STANDARD

Cosmetics — Microbiology — General instructions for microbiological examination

This national standard is the identical implementation of ISO 21148:2017, and is adopted with the permission of the International Organization for Standardization.

Published by the South African Bureau of Standards
1 Dr Lategan Road Groenkloof ✉ Private Bag X191 Pretoria 0001
Tel: +27 12 428 7911 Fax: +27 12 344 1568
www.sabs.co.za
© SABS

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. 014/009/26-295, valid until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

This page has been left blank intentionally

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. 014/009/26-295, valid until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.



COPYRIGHT PROTECTED DOCUMENT

© SABS

In terms of the Standards Act 8 of 2008, the copyright in all South African National Standards or any other publications published by the SABS Standards Division, vests in the SABS. Any use of South African National Standards is limited to use specifically prescribed by the SABS. In the case of a South African National Standard based on an international standard, ownership of the copyright vests in the organization from which the SABS adopted the standard, whether it be under licence or membership agreement. The SABS is obliged to protect such copyright and is authorized to make the relevant international organization aware of any misuse thereof. Unless exemption has been granted, no extract or full text of any South African National Standard may be copied, reproduced, stored in a retrieval system or transmitted in any form or by any means without prior written permission from the SABS Standards Division. This does not preclude the free use, in the course of implementing the standard, of necessary details such as symbols, and size, type or grade designations. If these details are to be used for any purpose other than implementation, prior written permission must be obtained.

Details, advice and limitations of use can be obtained from the Manager: Standards Sales and Information Services. Tel: +27 (0) 12 428 6883 email: sales@sabs.co.za

SABS – Standards Division

The objective of the SABS Standards Division is to develop, promote and maintain South African National Standards. This objective is incorporated in the Standards Act, 2008 (Act No. 8 of 2008).

The SABS continuously strives to improve the quality of its products and services and would therefore be grateful if anyone finding an inaccuracy or ambiguity while using this standard would inform the secretary of the technical committee responsible, the identity of which can be found in the foreword.

Buying Standards

Contact the Sales Office for South African and international standards, which are available in both electronic and hard copy format. Tel: +27 (0) 12 428 6883 email: sales@sabs.co.za

South African National Standards are also available online from the SABS Webstore www.store.sabs.co.za

Information on Standards

SABS Customer Services provide comprehensive standards-related information on national, regional and international standards. Tel: +27 (0) 12 428 7911 / 0861 27 7227 email: info@sabs.co.za

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. 014/009/26-295, valid until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

SANS 21148:2020
Edition 2
ISO 21148:2017
Edition 2

Table of changes

Change No.	Date	Scope

National foreword

This South African standard was prepared by National Committee SABS/TC 217, *Cosmetics*, in accordance with procedures of the South African Bureau of Standards, in compliance with annex 3 of the WTO/TBT agreement.

This document was approved for publication in August 2020.

This document supersedes SANS 21148:2008 (edition 1).

Compliance with this document cannot confer immunity from legal obligations.

© SABS

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SABS/DDS/2019/0[illegible] until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution or access outside approved networks is prohibited.

INTERNATIONAL STANDARD

ISO 21148

Second edition
2017-06

Cosmetics — Microbiology — General instructions for microbiological examination

Cosmétiques — Microbiologie — Instructions générales pour les examens microbiologiques

Reference number
ISO 21148:2017(E)

© ISO 2017

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/2019/0[illegible] until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.



COPYRIGHT PROTECTED DOCUMENT

© ISO 2017, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

Contents

Page

Foreword ..... **v**

Introduction ..... **vi**

1 Scope **1**

2 Normative references **1**

3 Terms and definitions **1**

4 Premises **1**
4.1 Test areas 1
4.2 Additional areas 2
4.3 Location of the premises 2
4.4 Equipping the premises 2
4.5 Maintenance 3

5 Equipment **3**
5.1 General 3
5.2 Microbiological cabinets 3
5.3 Balances 3
5.4 Homogenizer 4
5.5 pH–meter 4
5.6 Autoclave 4
5.7 Incubator 4
5.8 Water baths 4
5.9 Refrigerator or cold-storage room 4
5.10 Freezer 4
5.11 Sterilizing oven 5
5.12 Colony-counting device 5
5.13 Other equipment 5

6 Strains of microorganisms **5**

7 Personnel **6**
7.1 Competence 6
7.2 Hygiene 6

8 Preparation of the apparatus and glassware **6**
8.1 Preparation 6
8.2 Sterilization 6
8.2.1 Sterilization by dry heat 6
8.2.2 Sterilization by moist heat 7
8.3 Disposable apparatus 7
8.4 Management of clean apparatus and glassware 7
8.5 Management of sterile apparatus and glassware 7
8.6 Treatment of contaminated material 7
8.7 Washing 7

9 Preparation and sterilization of culture media and reagents **8**
9.1 General 8
9.2 Water 8
9.3 Preparation of culture media 8
9.3.1 General 8
9.3.2 Rehydration 8
9.3.3 Measurement of pH 8
9.3.4 Dispensing 8
9.4 Sterilization 9
9.4.1 General 9
9.4.2 Sterilization by moist heat 9
9.4.3 Sterilization by filtration 9

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/2019/0... until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

9.5 Storage ... 9
9.5.1 General ... 9
9.5.2 Laboratory-prepared culture media and reagents ... 9
9.5.3 Ready-to-use culture media and reagents ... 10
9.6 Melting of agar culture media ... 10
9.7 Preparation of Petri dishes ... 10

10 Laboratory samples ... 10
10.1 General ... 10
10.2 Sampling the cosmetic product ... 10
10.3 Transport ... 10
10.4 Receipt and storage ... 11
10.5 Handling products and samples ... 11
10.6 Conservation and destruction of products ... 11

11 Operating practices ... 11
11.1 Hygienic precautions during the testing ... 11
11.2 Preparation of the initial suspension and of sample dilutions ... 12
11.2.1 General ... 12
11.2.2 Water-miscible product ... 12
11.2.3 Water-immiscible products ... 13
11.3 Counting methods ... 13
11.4 Detection methods ... 13

12 Expression of results ... 13

13 Neutralization of the antimicrobial properties of the product ... 13

Annex A (informative) **Basic identification techniques ... 14**

Annex B (informative) **Basic techniques for counting and plating ... 19**

Annex C (informative) **Preparation and calibration of inoculums ... 20**

Bibliography ... 21

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. CA/INDS/2196 valid until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

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 217, *Cosmetics*.

This second edition cancels and replaces the first edition (ISO 21148:2005), of which it constitutes a minor revision.

It also incorporates the Technical Corrigendum ISO 21148:2005/Cor 1:2006.

The following changes have been made:

a) in the Introduction, "validated" was changed to "demonstrated to be suitable";

b) in Clause 6, "validation of the methodology" was changed to "verification of the methods' suitability";

c) in 8.2.1, "validated" was changed to "demonstrated to be suitable";

d) in Clause 13, "validated" was changed to "demonstrated";

e) in A.5, "validated" was changed to "demonstrated to be suitable";

f) in B.3, editorial changes were applied.

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/21148:2020 valid until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

Introduction

The purpose of this document is to help ensure that the general techniques used for conducting cosmetic microbiological examinations are the same in other laboratories that adopt these standards, to help achieve homogeneous results in different laboratories and to contribute towards the protection of the health of the laboratory personnel by preventing risk of infection.

When conducting microbiological examinations for cosmetic products, it is especially important that:

— only those microorganisms which are present in the samples be isolated or enumerated;

— the microorganisms do not contaminate the environment.

In order to achieve this, it is necessary to pay attention to personal hygiene and to use working techniques which ensure, as far as possible, exclusion of extraneous contamination.

Since, in this document, it is possible to give only a few examples of the precautions to be taken during microbiological examinations, a thorough knowledge of the microbiological techniques and of the microorganisms involved is essential. It is important that the analyses be conducted as accurately as possible, including calculation of the number of microorganisms.

A large number of manipulations can, for example, unintentionally lead to cross-contamination and the analyst should always verify the accuracy of the results given by his/her technique. It is necessary to take special precautions, not only for reasons of hygiene, but also to ensure good reproducibility of the results. It is not possible to specify all the precautions to be taken in all circumstances, but this document at least provides the main measures to be taken when preparing, sterilizing and storing the media and the equipment.

The given recommendations will allow enumeration and detection of mesophilic microorganisms which may grow under aerobic conditions.

The recommendations are applicable to the determination of the absence of, or limited occurrence of specified microorganisms that are of interest for cosmetic products.

The test methods are described in the individual standards. Alternative microbiological procedures can be used provided that their equivalence has been demonstrated or the method has been otherwise demonstrated to be suitable. The choice of a specific method, or combination of methods mentioned in these International Standards will depend on the purpose for performing the test and it is for the user to decide which approach is best for his/her application.

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. CLA/ISO/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

Cosmetics — Microbiology — General instructions for microbiological examination

1 Scope

This document gives general instructions for carrying out microbiological examinations of cosmetic products, in order to ensure their quality and safety, in accordance with an appropriate risk analysis (e.g. low water activity, hydro-alcoholic, extreme pH values).

Because of the large variety of products and potential uses within this field of application, these instructions might not be appropriate for some products in every detail (e.g. certain water-immiscible products).

2 Normative references

There are no normative references in this document.

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
product
portion of an identified cosmetic product received in the laboratory for testing

3.2
sample
portion of the *product* (3.1) (at least 1 g or 1 ml) that is used in the test to prepare the *initial suspension* (3.3)

3.3
initial suspension
suspension (or solution) of the *sample* (3.2) in a defined volume of an appropriate enrichment broth

3.4
sample dilution
dilution of the *initial suspension* (3.3)

4 Premises

4.1 Test areas

The areas required for the specific operation of a microbiology laboratory are as follows:

— receipt, storage, preparation and processing of the samples;

— preparation and sterilization of culture media, apparatus and glassware;

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/21148:2020 valid until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

— performance of analyses: weighing, dilutions, inoculations, subculturing, incubation, maintenance of the strain, etc.;

— decontamination and cleaning of apparatus, glassware and processing of the analysis waste.

4.2 Additional areas

The areas included in this category are, for example:

— entrances, corridors, stairways, lifts;

— administrative areas (e.g. secretarial, offices, documentation rooms, etc.);

— cloakrooms and toilets;

— archive rooms;

— stores.

4.3 Location of the premises

The environment within which the microbiological analyses are carried out shall not affect the reliability of the analyses.

Care shall be taken to locate the premises so as to avoid risk of cross-contamination.

Care shall be taken to ensure protection against extreme conditions such as excess temperature, dust, humidity, steam, noise, vibration, exposure to direct sunlight, etc.

The surface area shall be sufficiently large to keep the work areas clean and orderly.

During the course of the tests, care shall be taken to limit access to the test areas to only those persons required to conduct the tests.

Separate rooms and/or separate areas and/or specific enclosures should be provided for the following:

— receipt, storage and preparation of samples;

— manipulation of microbial cultures;

— preparation of culture media, apparatus and glassware;

— decontamination and washing area;

— sterilization;

— incubators, refrigerators and freezers.

4.4 Equipping the premises

4.4.1 The test premises shall be fitted out in the following ways in order to reduce the risks of contamination by dust and therefore by microorganisms:

— walls, ceilings and floors should be smooth, non-porous, easy to clean and resistant to detergents and disinfectants used in laboratories;

— overhead pipes conveying fluids should not cross the premises unless they are hermetically enclosed;

— sun-protection systems, when used, shall be installed on the outside of the windows, where practicable;

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/2019/0001 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

— windows and doors shall be able to be closed when conducting the test in order to minimize draughts. Furthermore, they shall be designed so as to avoid the formation of dust traps and hence, to facilitate the cleaning.

4.4.2 The ambient temperature and air quality (microorganism content, humidity, dust-spreading rate, etc.) shall be compatible with carrying out the tests.

According to needs, a filter-ventilation and/or a microbiological cabinet are recommended for this purpose.

4.4.3 The laboratory bench tops and furniture shall be made of smooth, non-porous impermeable materials, which are easy to clean and disinfect. Cabinet and equipment tops should be accessible for cleaning.

Non-fixed laboratory furniture shall be designed so as to facilitate cleaning the floors.

It is desirable that documents or books that are not frequently used be kept outside the test areas.

4.5 Maintenance

The floors, walls, ceiling, laboratory bench tops and furniture shall be maintained in good order to avoid cracks where dirt might particularly accumulate and thus cause a source of contamination.

Regular cleaning and, when relevant, disinfection shall be carried out in order to keep the premises in a condition suitable for conducting tests.

The ventilation systems and their filters shall be regularly maintained and filters changed when necessary.

5 Equipment

5.1 General

In general, all equipment shall be kept clean and in proper working condition.

Maintenance operations should be monitored. The measurement instruments and apparatus shall be regularly verified according to an appropriate timetable and results recorded.

5.2 Microbiological cabinets

Cabinets are of two types:

a) clean-air cabinets, which are intended to protect the product from extraneous contamination and to minimize contamination due to the operator;

b) safety cabinets, which are intended to protect the product from extraneous contamination, and also to protect the operator and the environment.

Either cabinet can be used. Safety cabinets should be used for all work involving risk for the operator.

A cabinet is a dust-free workstation equipped with vertical laminar airflow. In microbiology, a safety cabinet is used to retain the microorganisms on filters.

5.3 Balances

A microbiology laboratory for analyses of cosmetic products should be equipped with balances of the required range and accuracy for the different products to be weighed. Generally, the accuracy required

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

for weighing the samples to be analysed and some components of the culture media and reagents is ±0,01 g.

5.4 Homogenizer

This equipment (e.g. blender, stomacher, etc.) may be used to prepare the initial suspension from the test samples of non-liquid products.

5.5 pH–meter

The pH-meter should be capable of measuring to an accuracy of ±0,1 pH units and its minimum measuring threshold shall be 0,01 pH units.

5.6 Autoclave

The autoclave shall be kept in good operating condition and shall regularly be inspected by the competent departments in accordance with the manufacturer's instructions and proper documentation should be recorded.

The autoclave shall not be used to sterilize both clean materials and also to decontaminate used materials at the same time. Wherever possible, separate autoclaves for these two processes should be used.

5.7 Incubator

Incubators shall be equipped with a regulation system which allows the temperature to be kept even and stable over their entire working volume.

If the ambient temperature is close to, or higher than, that of the incubator, use an incubator with a cooling system.

Incubators should be protected from direct sunlight.

If possible, incubators should not be completely filled in one single operation because the culture media will take a long time to equilibrate to temperature, whatever type of incubator is used (forced-air convection or otherwise).

The temperature shall be checked and recorded at least every working day.

5.8 Water baths

Water baths are of two types:

— thermostatically-controlled baths, suitable for incubation of inoculated culture media, for identification tests, etc.;

— temperature-controlled water baths for maintenance of sterile agar media in a molten state for later use in specified procedures.

The required temperature and accuracy are stipulated in each method of application.

5.9 Refrigerator or cold-storage room

The temperature, unless otherwise specified, shall be 5 °C ± 3 °C.

5.10 Freezer

The temperature, unless otherwise specified, shall be below –18 °C.

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. CLA/DDS/2019/1 valid until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

5.11 Sterilizing oven

A sterilizing oven is a chamber which allows the destruction of microorganisms by dry heat.

The temperature shall be evenly distributed within the chamber.

The oven shall be equipped with:

— a thermostat;

— a thermometer or a recording thermocouple;

— a duration indicator or a programmer/timer.

5.12 Colony-counting device

A colony-counting device may be used.

5.13 Other equipment

WARNING — Volumetric glassware shall not be sterilized in a sterilizing oven.

Other equipment and apparatus for everyday use include the following:

a) filtration apparatus (see below);

b) glass or plastic containers (test tubes, flasks, bottles);

c) glass or plastic Petri dishes (most commonly between 85 mm and 100 mm in diameter);

d) glass or plastic pipettes (10 ml, 2 ml, 1 ml), automatic pipettes;

e) sampling instruments;

f) wires and loops (of nickel/chromium, platinum/iridium or disposable plastic, etc.);

g) optical microscope;

h) gas burner or wire incinerator;

i) dispenser for culture media and reagents;

j) mechanical stirrer.

If the membrane filtration method is used, the equipment shall also include:

— a membrane filtration system or filtration apparatus constructed of a suitable material, with a filter holder of at least 50 ml, and suitable for use of filters with diameter 47 mm to 50 mm and not more than 0,45 µm pore size;

— the type of membrane material is chosen in such a way that the bacteria are not affected by the residual components of the sample to be investigated;

— a vacuum source able to give an even filtration flow rate (the device shall be set as to obtain the filtration of 100 ml of liquid in less than 2 min).

6 Strains of microorganisms

The strains needed for the verification of the methods' suitability are indicated in each method of application.

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

7 Personnel

7.1 Competence

All personnel working in a microbiology laboratory shall have received adequate training to enable them to conduct properly the operations entrusted to them.

The personnel who perform the tests shall have a good knowledge and sufficient practical experience with microbiological techniques and the microorganisms sought. The person in charge shall be able to interpret the accuracy and precision required to yield acceptable results.

7.2 Hygiene

In the field of personal hygiene, the following precautions shall be taken not only in order to avoid contaminating the samples and culture media, but also in order to avoid risk of personnel infection:

— wear laboratory clothing that is light-coloured, clean and in good condition, manufactured from a fabric which limits the risks of flammability; this clothing shall not be worn outside the work areas;

— keep nails perfectly clean and well groomed, and preferably short;

— wash hands, before and after microbiological examinations and immediately after visiting the toilets or eating; for drying hands, use disposable paper or disposable cloth towels;

— when inoculating, avoid speaking, coughing, etc.;

— do not smoke, drink or eat in the test areas;

— do not put food for personal consumption in the laboratory refrigerators;

— special precautions shall be taken by persons having infections or illnesses that are likely to contaminate samples with microorganisms and may invalidate results.

8 Preparation of the apparatus and glassware

8.1 Preparation

The apparatus and glassware used in microbiology shall be prepared in such a manner as to guarantee its cleanliness and/or sterility up until the time of use.

Stopper tubes and cap bottles by appropriate material prior to sterilization.

Stopper the pipettes with cotton or any other appropriate material.

If necessary, the apparatus and glassware to be sterilized should be placed in special containers or wrapped in an appropriate material (e.g. special paper, aluminium, etc.).

8.2 Sterilization

8.2.1 Sterilization by dry heat

Heat in sterilizing oven for at least 1 h at 170 °C to 180 °C or use any combination of time and temperature if they are demonstrated to be suitable.

Indicators can be used in order to make certain that the sterilization has been achieved.

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

8.2.2 Sterilization by moist heat

Heat for at least 15 min at minimum of 121 °C in an autoclave. Indicators can be used in order to make certain that the sterilization has been achieved.

8.3 Disposable apparatus

Disposable apparatus may be used in the same way as the re-usable glassware (Petri dishes, pipettes, tubes, etc.) if the specifications are similar.

In this case, contact the manufacturer to determine that the proposed apparatus and glassware is suitable for use in microbiology (in particular sterility) and that the material contains no substances that inhibit the growth of microorganisms.

Disposable apparatus shall be decontaminated prior to its disposal. Other than the methods described in 8.6, and depending upon national regulations, incineration may be used. If there is an incinerator on the premises, decontamination and disposal may be achieved in a single operation.

8.4 Management of clean apparatus and glassware

Clean apparatus and glassware shall be protected against external contamination during storage, under conditions which maintain their cleanliness.

8.5 Management of sterile apparatus and glassware

Prior to use, the apparatus and glassware shall be stored under conditions which allow them to remain sterile. Disposable apparatus and glassware shall be stored in accordance to the manufacturer's specifications, without any damage to the packaging; laboratory-prepared apparatus and glassware shall be stored in clean containers.

When sterilizing apparatus and glassware intended for microbiology, an expiry date (or a manufacturing date) shall be put on each packaging. Hermetically sealed apparatus and glassware can be stored for up to 3 months prior to use unless otherwise specified.

8.6 Treatment of contaminated material

After use (culture of microorganisms or material in contact with microorganisms), the apparatus and glassware and their contents shall be treated for destruction of microorganisms, prior to cleaning or disposal, whatever the microorganism involved.

According to the nature of the materials, disinfection (see 11.1), sterilization (see 8.2) or incineration of disposable material (see 8.3) may be used.

8.7 Washing

Wash apparatus and glassware after they have been treated (see 8.6).

Empty the containers of their contents.

Prior to washing, separate seals from stoppers or caps, as appropriate.

Rinse detergent residue from equipment with tap water. Rinse clean equipment with water (see 9.2).

In the absence of any commercial product, a sodium carbonate solution of 0,125 % (mass fraction) can be used, followed by immersion in diluted acid [e.g. hydrochloric acid c(HCl) = 0,1 mol/l].[2]

Specialized apparatus and glassware may be used in order to facilitate cleaning operations (e.g. pipette washers, dishwashers, ultrasonic baths, etc.).

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/ISO/21148:2020 valid until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

9 Preparation and sterilization of culture media and reagents

9.1 General

The accurate preparation of culture media is one of the fundamental steps in microbiological analysis and it shall be given special care.

9.2 Water

WARNING — Water processed through an ion exchanger (deionized) may have a high microorganism content; it is therefore advisable not to use such water without verifying that the microorganism content of the water is low. Consult the manufacturer for the best way to minimize microbial contamination. Heavily contaminated deionized water that has been filter sterilized may still contain substances inhibitory to the growth of some microorganisms.

Use distilled water or water of equivalent quality, i.e. purified water[7][9][11] or deionized water[3]. If the distilled water is prepared from chlorinated water, neutralize the chlorine prior to the distillation.

The water shall be stored in containers manufactured of inert materials (e.g. neutral glass, polyethylene, etc.).

9.3 Preparation of culture media

9.3.1 General

Two types of culture media preparation exist:

— from basic ingredients, dehydrated or not; or

— from dehydrated complete media.

Follow the manufacturer's specified storage conditions and expiration date.

Do not use the culture media beyond the stated shelf life.

Protect laboratory culture media that is in dehydrate from absorbing additional moisture from the environment during storage and use.

9.3.2 Rehydration

Follow the manufacturer's recommendation for rehydration.

9.3.3 Measurement of pH

Measure the pH using a pH-meter (5.5) and adjust it, if necessary, so that, after sterilization and cooling down to room temperature, the medium is at the required pH ±0,2 units, unless otherwise stated.

NOTE The adjustment is normally carried out using a solution of approximately 40 g/l (about 1 mol/l) of sodium hydroxide (NaOH) or approximately 36,5 g/l (about 1 mol/l) of hydrochloric acid (HCl).[2]

9.3.4 Dispensing

Dispense the medium into appropriate containers, either manually or using automatic apparatus.

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/ISO/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

9.4 Sterilization

9.4.1 General

The sterilization of culture media and reagents can be carried out using various techniques including:

— sterilization by moist heat;

— sterilization by filtration.

According to the technique used, once sterilized, the media should be monitored, in particular with respect to pH, colour, sterility and microbiological performance.

9.4.2 Sterilization by moist heat

Use an autoclave (5.6) for sterilization. Generally, the sterilization step takes 15 min or longer at 121 °C. Adapt the sterilization cycle as necessary for the volume and number of containers, the loading pattern and the media type.

The performance of the sterilization shall be verified using appropriate means.

9.4.3 Sterilization by filtration

Sterilization by filtration can be performed under vacuum or at pressurized conditions.

Use sterile membranes and filter elements with a pore diameter of 0,22 µm (except in certain cases, where 0,45 µm can be used). Refer to the manufacturer's instructions regarding the use of filter elements or membranes which have been purchased in a sterile condition.

Sterilize the different components of the filtration apparatus, assembled or not, in the autoclave for 15 min at 121 °C. If necessary, aseptic assembly can be performed in a microbiological cabinet after autoclaving. Certain appliances can be purchased in a sterile condition.

9.5 Storage

9.5.1 General

Each package of bottles, tubes and Petri dishes shall be labelled and shall bear the following details:

— name of the medium;

— date of preparation and/or expiry date.

9.5.2 Laboratory-prepared culture media and reagents

Culture media dispensed in tubes or bottles and reagents which are not used immediately shall be protected against light and desiccation using appropriate inert stoppers or screw-on lids with inert liners.

They shall be kept under conditions which prevent their composition from being modified.

Never use media which have become dehydrated.

Prior to use, it is desirable that the temperature of the culture media be equilibrated to that of the laboratory, unless otherwise specified.

EXAMPLE TSA medium, prepared in the laboratory, is to be kept in flasks and stored in the dark, generally for no more than 2 months, unless otherwise specified.

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

9.5.3 Ready-to-use culture media and reagents

It is necessary to comply with the manufacturer's instructions:

— expiry date;

— storage temperature and conditions;

— conditions for use (pH, etc.);

— efficiency control.

9.6 Melting of agar culture media

Melt a culture medium by placing it in boiling water bath or by any other process which gives identical result (e.g. a steam flow-through autoclave).

Avoid over-heating and remove the culture medium as soon as it has melted. Keep the culture medium in a molten state in a water bath kept at no more than 48 °C until such time as it is to be used. Never use culture medium at a temperature higher than 48 °C. It is preferable not to keep a molten medium more than 8 h. In the case of particularly sensitive culture media, the melting duration shall be shortened.

No unused medium shall be resolidified for subsequent use.

9.7 Preparation of Petri dishes

Pour the molten agar culture medium into sterile Petri dishes so as to obtain a thickness of at least 3 mm to 4 mm (e.g. for 90 mm diameter dishes, 15 ml to 20 ml of agar medium are normally required).

Allow the agar medium to cool and solidify by placing the Petri dishes on a cool, horizontal surface.

Use the thus-prepared Petri dishes immediately or store them under (in the dark and in the appropriate temperature and duration) conditions which prevent their composition from being modified. Label the dishes as described in 9.5.

Drying may be necessary before use.

Ready-prepared agar medium plates are available commercially. Store and use them according to the manufacturer's instructions.

10 Laboratory samples

10.1 General

Product and sample are defined in 3.1 and 3.2, respectively.

10.2 Sampling the cosmetic product

It is important that the laboratory receive a product which is truly representative of the cosmetic product and has not been damaged during transport or storage.

Sampling shall have been carried out in accordance with the specific appropriate documents. If there is no specific document, it is recommended that the parties concerned come to an agreement on this subject.

10.3 Transport

During their transportation to the laboratory, products to be tested shall be kept under the conditions which minimize changes in the microbial content.

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. CLA/DDS/2196 valid until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

10.4 Receipt and storage

The laboratory personnel shall check the condition of the products on receipt and confirm that condition and quantity is satisfactory. If their condition is unsatisfactory or if their quantity is insufficient, the laboratory cannot perform the prescribed test.

However, in special circumstances if the test is completed, the personnel shall record the condition of the product and the reason for testing.

The products admitted into the laboratory shall be documented in such a manner that their progress through to the time of drafting the test report can be monitored.

The following information shall be noted:

— date of receipt;

— characteristics of the sampling operation (sampling date, sampling conditions, etc.);

— name, reference, origin and requesting party;

— characteristics of the product.

If necessary, store products to be tested at room temperature. Do not incubate, refrigerate or freeze products and samples before or after analysis.

10.5 Handling products and samples

In order to avoid contamination of the environment and of the products and samples, handle the products in such a manner as to avoid any risk of contamination. To achieve this, follow aseptic techniques including, for example:

— any instrument that is used to open the packaging shall be sterile;

— any instrument that is used to remove the sample from the product shall be sterile;

— if required, sanitize the package and cover to be opened as appropriate.

10.6 Conservation and destruction of products

Except for special cases, keep the products until all results have been obtained, or longer if necessary.

Discard products from which samples have been taken, except if the nature and the level of contamination justify that they be treated as a contaminated material (see 8.6).

11 Operating practices

11.1 Hygienic precautions during the testing

Precautions shall be taken in order to conduct the work as far as possible under aseptic conditions, for example:

a) make sure that the work area is clean and that there are no draughts (doors and windows closed);

b) before and after the work, decontaminate the work surface with an appropriate disinfectant;

c) prior to starting, make sure that everything required for carrying out the work is available;

d) in the case of work conducted in a microbiological cabinet, use sterile gloves or decontaminate hands prior to starting work and avoid crossing forearms and hands;

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/21148:2020 valid until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

e) when not working in a microbiological cabinet, open the sample container in the vicinity of a flame, holding them in the most inclined position possible;

f) carry out the work as quickly as possible without making any unnecessary movements;

g) if the total contents of a bag of disposable pipettes, Petri dishes, etc., is not used during the course of an examination, make sure that the container is properly closed after taking the appropriate number of units;

h) sterilize loops and inoculation wires, etc., before and after use with a flame; in order to avoid splatter of substances and microorganisms, preferably use a wire incinerator whenever possible or use disposable sterile loops and wires;

i) place used pipettes, spatulas, etc., in specific receptacles containing an appropriate disinfectant (e.g. a sodium hypochlorite solution for the pipettes) prior to treatment (see 8.6);

j) place reusable equipment which may contain microorganisms into specific containers prior to sterilization, before the washing operation;

k) place used disposable apparatus in appropriate containers prior to sterilization or incineration (see 8.6);

l) immediately mop up spillage by means of cotton pads or any other appropriate material impregnated with appropriate disinfectant, then clean and disinfect the work surface prior to continuing.

The manipulation of products and subsequent cultures likely to contain pathogenic bacteria requires special precautions. The following are recommended:

— a microbiological cabinet for all manipulations required for conducting the analysis;

— automatic pipettes (pipetting by mouth suction is strictly prohibited).

NOTE Droplets are a major cause of environmental contamination and of infection. Droplets can be formed for example:

— when using shakers, syringes, etc.;

— when emptying pipettes by blowing;

— when sterilizing wet inoculation loops or needles.

It is therefore necessary to minimize their formation.

11.2 Preparation of the initial suspension and of sample dilutions

11.2.1 General

In the case of the preparation of an initial suspension and of sample dilutions, the time that elapses between the end of the preparation and the moment the inoculum comes into contact with the culture medium shall not exceed 45 min, unless specifically mentioned in the relevant documents.

The initial suspension is prepared from a sample of at least 1 g or 1 ml of the well-mixed product under test.

Record the exact mass or volume of the sample (S).

11.2.2 Water-miscible product

Transfer the sample (S) of product into any suitable container and make a precise and appropriate dilution according to the standard to be applied.

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/ISO/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

Record the dilution factor (d).

11.2.3 Water-immiscible products

Transfer the sample (S) of product into any suitable container containing an appropriate amount of solubilizing agent (e.g. polysorbate 80) and make a precise and appropriate dilution according to the standard to be applied.

Record the dilution factor (d).

11.3 Counting methods

Follow the specifications of the standard method to be applied. For information, see Annex B.

11.4 Detection methods

Follow the specifications of the standard method to be applied.

12 Expression of results

Follow the specifications of the standard method to be applied.

13 Neutralization of the antimicrobial properties of the product

Prior to detection or enumeration viable microorganisms in a cosmetic product, the possible inhibition of microbial growth by the sample shall be neutralized. In all cases and whatever the methodology, the neutralization of the antimicrobial properties of the product shall be checked and demonstrated.

Follow the specifications of the standard method to be applied.

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

Annex A
(informative)

Basic identification techniques

A.1 Preparation of a pure culture

A.1.1 General

Begin the preparation of a pure culture by the selection of a colony on or in an agar medium which has been inoculated with a dilution of the test sample or with a culture.

Then inoculate the selected colony on a non-selective agar culture medium. After incubation, select a well-isolated colony. Repeat the operation if necessary.

Use the plating-out techniques described in A.1.2. Different methods may prove to be necessary in specific cases.

A.1.2 Plating out

A.1.2.1 General

Take a small quantity from the surface of a well-isolated colony using the tip of a sterile loop.

Then plate out, either directly with the cells present on the loop (see A.1.2.2) or after having prepared a suspension of these cells (see A.1.2.3).

A.1.2.2 Direct method: Example

Using the tip of the loop, inoculate, in close streaks, a portion of about one-third of the agar medium surface area. Sterilize and cool down the loop. From the edge of the inoculated area, make another series of streaks, less close together than in the first case, over half of the surface area not yet inoculated. Repeat the operation over the remaining surface area making more-spread-out streaks (see Figure A.1).

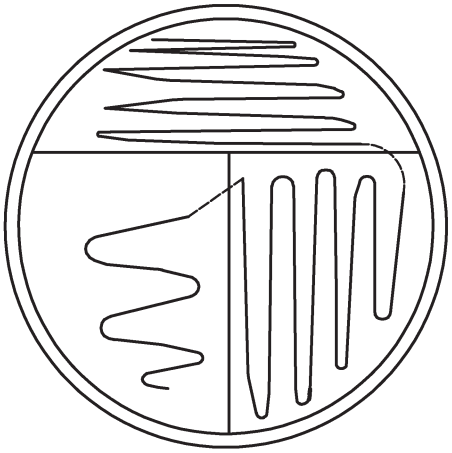


Figure A.1 — Example of plating out: Direct method

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

A.1.2.3 Method using dilution

Suspend the cells in 1 ml to 2 ml of the selected dilution, rubbing the inoculated loop against the wall of the tube at the surface of the liquid, then mix well.

Sterilize and cool down the loop. Using the loop, take a small portion of the microbial suspension and proceed as stated in A.1.2.2.

A.1.3 Incubation

Invert the inoculated Petri dishes and place them in the incubator for the chosen time at the chosen temperature.

A.1.4 Selection

After incubation, select a well-isolated colony from the dish, either for subsequent plating out, or for the tests to be performed.

If possible, the final tests should be carried out using cells stemming from one single colony. If there is insufficient cell material in one colony, it should first be subcultured in a liquid medium or on the agar medium slant, after which the subculture can be used for the tests to be performed.

A.2 Gram's stain (modified Hucker technique)

A.2.1 General

This staining of bacterial cells allows description for the morphology of the bacteria and classification of them into two groups as a function of whether or not they are capable of retaining the violet stain of crystal violet under the test conditions. This division results mainly from differences in the structure of the cell walls of the two groups and it is correlated with other major differences between the two groups. There are a number of ways to conduct a Gram's stain, but all follow the sequences given below.

A.2.2 Solutions

A.2.2.1 General

Commercially available solutions may be used. In this case, follow the manufacturer's recommendations.

A.2.2.2 Crystal violet solution

A.2.2.2.1 Composition

Crystal violet	2,0 g
Ethanol (95 %)	20 ml
Ammonium oxalate ($C_2H_8N_2O_4$)	0,8 g
Water	80 ml

A.2.2.2.2 Preparation

Dissolve the crystal violet in the ethanol and the ammonium oxalate in the distilled water. Mix the two solutions and allow the mixture to stand for 24 h prior to use.

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

A.2.2.3 Iodine solution

A.2.2.3.1 Composition

Iodine	1,0 g
Potassium iodide (KI)	2,0 g
Water	100 ml

A.2.2.3.2 Preparation

Dissolve the potassium iodide in 10 ml of distilled water, add the iodine in fractions.

After dissolution, make up to 100 ml in a volumetric flask.

A.2.2.4 Safranin solution

A.2.2.4.1 Composition

Safranin O	0,25 g
Ethanol (95 %)	10 ml
Water	100 ml

A.2.2.4.2 Preparation

Dissolve the safranin in the ethanol then mix with the distilled water. Make up volumetrically to a final volume of 100 ml.

When crystal violet is used, the stability of the solution should be verified. For the verification, mix one drop of the crystal violet solution with one drop of iodine solution on a glass slide to see a chemical reaction. If the crystallization is seen on the glass slide, do not use the crystal violet solution.

A.2.2.5 Staining technique

After fixing (e.g. with a flame) the bacterial film on the microscope slide prepared from a culture 18 h to 24 h, or when the broth is turbid, cover the film with the crystal violet solution (see A.2.2.2). Allow it to react for 1 min.

Gently rinse the inclined slide with water for a few seconds.

Cover the slide with the iodine solution (see A.2.2.3). Allow it to react for 1 min. Gently rinse the inclined slide with water for a few seconds.

Pour gently and continuously a film of ethanol (95 %) onto the inclined slide over a period of no more than 30 s and until no more of the violet colour is emitted.

Gently rinse the inclined slide with water in order to eliminate the ethanol.

Cover the slide with the solution of safranin (see A.2.2.4) for 10 s.

Gently rinse the inclined slide with water.

Dry the slide.

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

A.2.2.6 Interpretation

Examine the slide under the high-power objective of the microscope (5.13). Those bacterial cells which appear blue or violet are termed Gram-positive; those which are coloured dark pink to red are termed Gram-negative.

For a pure culture of certain bacterial types, both Gram-positive and Gram-negative cells can be obtained in a same microscope field.

NOTE Densely packed cells can give an uncharacteristic response.

A.3 Test for catalase

A.3.1 General

The detection of this enzyme, which decomposes hydrogen peroxide (H_2O_2) into water and oxygen, can be carried out using a broth culture, an agar culture or one single colony on an agar medium.

A.3.2 From a broth culture

Add to 1 ml of the culture, 0,5 ml of a 10-volume [3 % (mass fraction)] hydrogen peroxide solution. Observe the occurrence of oxygen bubbles (catalase positive) or absence (catalase negative).

A.3.3 From an agar medium culture

Cover the culture with 1 ml to 2 ml of a 10-volume [3 % (mass fraction)] hydrogen peroxide solution.

Observe immediately and after 5 min whether or not oxygen bubbles have formed.

A.3.4 From a colony

Place separately two drops of a 10-volume hydrogen peroxide solution on a microscope slide.

Pick off a colony with a sterile glass or plastic rod (especially not a metallic wire) and gently emulsify it in one of the two drops. Observe immediately and over several minutes (at least 1 min) whether or not oxygen bubbles have formed. In the event of doubt, cover each of the drops with a cover slide and compare the occurrence of bubbles under both cover slides.

The observation can be conducted macroscopically or using a low-magnification microscope.

A.4 Test for oxidase

A.4.1 General

The detection of oxidase is carried out by the change in colour of a compound at the time of oxidation under the action of this enzyme.

A.4.2 Reagent

A.4.2.1 Composition

N,N,N′,N′-Tetramethyl-3-*p*-phenylenediamine dihydrochloride ($C_{10}H_{16}N_2{\cdot}2HCl$)	1,0 g
Water	100 ml

A.4.2.2 Preparation

Dissolve the reagent in cold water. Prepare the reagent immediately prior to use.

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/2019/0... until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

Commercially available disks or sticks may be used. In this case, follow the manufacturer's recommendations.

A.4.2.3 Technique

Moisten a piece of filter paper with the reagent. Take a sample of the bacterial culture obtained from an agar medium using a platinum wire or a glass or plastic rod (a nickel/chrome wire gives false positive) and deposit it on the moistened filter paper.

A.4.2.4 Interpretation of the result

In the case of the presence of oxidase, a violet to purple colour appears within a period of between 5 s and 10 s. If the colour has not changed after 10 s, the test is considered as being negative.

A.5 Use of biochemical tests for identification

Currently available biochemical tests may be used for identification. However, all commercialized tests do not present the same level of reliability. Their performance shall therefore be assessed before use, except if they have been demonstrated to be suitable by the manufacturer and/or an independent organization.

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/DDS/2019/0... until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

Annex B
(informative)

Basic techniques for counting and plating

B.1 Inoculation for poured plates

Prepare the medium, the Petri dishes, the diluents and the dilutions to be examined in quantities and numbers corresponding to the inoculation plan.

Dispense the defined volumes of the dilutions to be examined into the Petri dishes (labelled). Pour the volume of the medium specified in 9.7 into each dish. Immediately mix the molten medium and the inoculum carefully so as to obtain a homogeneous distribution of the microorganisms within the mass of the medium. Allow to cool and solidify by placing the Petri dishes on a cool horizontal surface (the solidification time of the agar shall not exceed 10 min).

B.2 Surface inoculation

Deposit the inoculum in the centre of the labelled Petri dish onto the agar culture medium (prepared in accordance with 9.7). Spread it uniformly and as quickly as possible on the surface of the medium using a sterile glass or plastic spreader or rotate the dish until there is no longer any liquid visible on the agar surface.

B.3 Membrane filtration

Transfer a suitable amount of the sample, prepared according to the instructions given in each method, in the filtration apparatus wetted with a small volume of an appropriate sterile diluent. Filter immediately and wash according to the suitable procedure.

Transfer the membrane filter in a Petri dish onto the surface of the agar medium.

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/ISO/21148:2020 valid until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

Annex C
(informative)

Preparation and calibration of inoculums

C.1 Culture of the reference strains

In order to improve the repeatability and the reproducibility of the results, it is recommended to use the third (at least the second) subculture grown on agar medium, obtained from the cultures kept and prepared as indicated in the EN 12353[5] for the preparation of the inoculum of bacteria. When using *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus,* the subcultures are performed at intervals of 18 h to 24 h. When using *Candida albicans*, the first subculture or the second subculture grown for 36 h to 48 h are suitable.

C.2 Preparation of the cell suspensions

Take 10 ml of diluent and place in a 100 ml sterile flask containing 5 g of glass beads. Transfer loopfuls of the cells harvested from the agar medium into the diluent. The cells should be suspended in the diluent by immersing the loop in the diluent and rubbing it against the side of the flask to dislodge the cells. Shake the flask for 2 min to 3 min (using, if possible, a mechanical shaker). Aspirate the upper part of the suspension (avoiding any contact with the glass beads) and transfer the obtained suspension in a sterile container.

C.3 Calibration of the suspensions

Adjust the number of cells in the suspension to 1×10^8 cfu/ml to 3×10^8 cfu/ml (with *C. albicans*, to 1×10^7 cfu/ml to 3×10^7 cfu/ml) using the diluent and according to calibration data produced in the laboratory. For example, use a spectrophotometer [(620 ± 20) nm wave length] and disposable 10 mm path length cuvette. Measure the absorbance of an aliquot part of the suspension and, if necessary, dilute the solution to bring the absorbance within a defined value. Suitable optical density values are found between 0,150 and 0,460 according to the strains.

© ISO 2017 – All rights reserved
This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.

© SABS. This standard may reside on internal network or intranet on a Single Site only and is exclusively available to The Cosmetic, Toiletry and Fragrance Association in accordance with copyright exploitation agreement no. SANS/ISO/21148:2020 until 2027-07-31. The single-site licence allows authorised staff member of The Cosmetic, Toiletry and Fragrance Association to make five (5) printouts of the standard and will expire after one year unless renewed. Unauthorised copying, sharing, distribution, or access outside approved networks is prohibited.

Bibliography

[1] ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

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

[3] ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

[4] ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

[5] EN 12353, *Chemical disinfectants and antiseptics — Preservation of test organisms used for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity*

[6] CTFA. *Microbiology Guidelines*, pub. Cosmetic, Toiletry and Fragrance Assn., ISBN 1-88261-32-8, 2001

[7] EP. *Microbiological examination of non-sterile products (2.6.12 to 2.6.13)*. European Pharmacopoeia, Fourth Edition, 2002

[8] FDA. *Bacteriological Analytical Manual.* U.S. Food and Drug Administration, Eighth Edition, 1995

[9] J.P 14:2001, *General tests — Microbial limit test,* pub. Japanese Pharmacopoeia

[10] JCIA. *Microbial test methods for cosmetics, pub*. Japanese Cosmetic Industry Association, 1997

[11] USP 28:2005, *Microbial limit test* ⟨61⟩, pub. U.S. Pharmacopoeia

© ISO 2017 – All rights reserved

This product is copyrighted by SABS, 1 Dr Lategan Road, Groenkloof, 0181, South Africa. All rights reserved.