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Microbiology — Cosmetics — Guidelines for the application of ISO standards on Cosmetic Microbiology

Microbiologie — Cosmétique — Lignes directrices pour l'application des normes ISO relatives à la microbiologie cosmétique

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Foreword

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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).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 217, *Cosmetics*.

Introduction

Every cosmetic manufacturer has a dual responsibility relative to the microbiological quality of its products.

— The first is to ensure that the product, as purchased, is free from the numbers and types of microorganisms that could affect product quality and consumer health. This is generally ensured by applying cosmetic good manufacturing practice (GMP) (see ISO 22716) during the manufacturing and packaging operations and, if necessary, by using **microbial content tests** on finished products.

— The second is to ensure that microorganisms introduced during normal product use will not adversely affect the quality or safety of the product. This is generally ensured by conducting **preservation efficacy tests** (or **challenge tests**) during the development stage of the new product.

In order to ensure product quality and safety for consumers, it is advisable that an appropriate microbiological risk analysis be performed to determine the types of cosmetic products to which this Technical Report would be applicable.

— Products considered to present a low microbiological risk are described in ISO 29621. These products identified as "hostile" and produced in compliance with GMP pose a very low overall risk to the user. Therefore, products that comply with the characteristics outlined in ISO 29621 do not require microbiological testing including both challenge test and end product testing.

— For those products which are not considered "hostile", the microbiological quality has to be assessed by conducting tests with appropriate methods. ISO TC 217 provides a comprehensive set of standards to assess the antimicrobial preservation of cosmetic products and the microbiological quality of finished products (methods and limits). Manufacturers can decide not to test if they can demonstrate that their products comply with those requirements specified in ISO 17516 and/or ISO 11930.

Microbiology — Cosmetics — Guidelines for the application of ISO standards on Cosmetic Microbiology

1 Scope

This Technical Report gives general guidelines to explain the use of ISO cosmetic microbiological standards depending on the objective (in-market control, product development, etc.) and the product to be tested.

This Technical Report can be used to fulfil the requirements of the ISO standard on microbiological limits (ISO 17516).

2 Terms and definitions

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

2.1
cosmetic formulation
preparation of raw materials with a qualitatively and quantitatively defined composition

2.2
cosmetic product
finished cosmetic product that has undergone all stages of production, including packaging in its final container for shipment

2.3
sample
one or more representative elements selected from a set to obtain information about that set

2.4.1
microbial content
<quantitative> estimated number of viable aerobic mesophilic microorganisms (bacteria, yeasts and moulds) within a cosmetic

2.4.2
microbial content
<qualitatative> detectable specified or non-specified microorganisms within a cosmetic sample

2.5
preservation of a cosmetic formulation
set of means used to avoid microbial proliferation in a cosmetic formulation

EXAMPLE Preservatives, multifunctional compounds, hostile raw materials, extreme pH, low water activity values, etc.

2.6
antimicrobial protection of a cosmetic product
ability of a cosmetic product to overcome microbial contamination that might present a potential risk to the user

Note 1 to entry: The overall antimicrobial protection includes preservation of the formulation, the specific manufacturing process and protective packaging.

2.7
microbiologically low-risk products
products whose environment denies microorganisms the physical and chemical requirements for growth and/or survival (hostile products)

Note 1 to entry: This category of low-risk products applies to microbiological contamination which may occur during manufacturing and/or use by the consumer.

Note 2 to entry: A product whose packaging prevents the ingress of microorganisms is considered a microbiological low-risk product during its use.

Note 3 to entry: The inclusion of preservatives or other antimicrobial compounds in a formulation by itself would not necessarily constitute a low-risk product.

2.8
microbiological Risk Assessment (low risk products)
evaluation of product characteristics to determine if that product may be subject to microbial contamination

Note 1 to entry: These characteristics include the composition of the product, the production conditions, packaging and a combination of these factors (see ISO 29621).

3 Microbial content

3.1 General requirements

There are eight International Standards dealing with the microbial content of cosmetic samples (see Annex A).

— ISO 16212

— ISO 17516

— ISO 18415

— ISO 18416

— ISO 21149

— ISO 21150

— ISO 22717

— ISO 22718

Additionally, ISO 21148 gives general instructions for carrying out microbiological examinations of cosmetic products.

Because of the large variety of cosmetic products within this field of application, the methods described in these International Standards may not be appropriate for some products in every detail (e.g. certain water immiscible products).

Other methods (e.g. automated) may be substituted for the tests presented here provided that their equivalence has been demonstrated or the method has been otherwise verified.

The possible inhibition of microbial growth by the sample should be neutralized to allow the detection of viable microorganisms. In all cases and whatever the methodology, the neutralization of the antimicrobial properties of the product should be checked and demonstrated.

The study of the neutralization of the antimicrobial activity should be performed when the tests have to be carried out on new products and whenever there is a change in the experimental conditions of the test.

The given International Standards will allow the following:

— the enumeration and detection of mesophilic microorganisms which may grow under aerobic conditions;

— the determination of the absence of specified microorganisms that are of interest for cosmetic products;

and, therefore, to estimate if the product under test complies with the requirements of the International Standard on microbiological limits (ISO 17516).

The test methods are described in the individual standards. The choice of a specific method, or combination of methods, will depend on the purpose for performing the test. It is up to the user to decide which approach is best for each application.

Depending on the expected level of contamination of the sample to be tested, two different approaches can be used (see Annex A).

— Quantitative tests (enumeration) are to be used when there is no information on the microbiological quality of the sample or if it is expected to be contaminated.

— Qualitative tests (detection) can be used if the sample is presumably free from microbial contamination (e.g. based on product history) and can be very useful from an economical and production time standpoint.

In the event where microorganisms are detected, the presence of specified microorganisms should be checked according to 3.3 or 3.4.

3.2 Enumeration of mesophilic microorganisms (bacteria, yeasts and moulds)

For bacteria, the enumeration method described in ISO 21149 involves enumeration of colonies on a non-selective agar medium. Enumeration of colonies can be performed by a plate count method or a filtration method using a specified culture medium incubated under defined conditions.

The results are expressed as follows:

> "[number of] aerobic mesophilic bacteria per gram or per millilitre of product, expressed as cfu/g or cfu/ml".

For yeasts and moulds, the method described in ISO 16212 involves enumeration of colonies by a plate count method or a filtration method using a specified culture medium with antibiotic incubated under defined conditions.

An alternative condition using the culture medium without antibiotic is proposed in ISO 16212.

The results are expressed as follows:

> "[number of] yeast and mould per gram or per millilitre of product expressed, as cfu/g or cfu/ml".

3.3 Detection of specified microorganisms

The detection of skin pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* may be relevant. The detection of other kinds of microorganisms might be of interest since these microorganisms (including indicators of fecal contamination e.g. *Escherichia coli*) suggest hygienic failure during the manufacturing process.

The methods are described in ISO 18416 (*C. albicans*), ISO 21150 (*E. coli*) ISO 22717 (*P. aeruginosa*), and ISO 22718 (*S. aureus*).

The main steps of the methods are the following:

— an enrichment using a non-selective broth medium incubated under defined conditions to increase the number of microorganisms while avoiding the risk of inhibition by the selective ingredients present in selective/differential growth media;

— then, if growth is detected, an isolation on a selective medium followed by identification tests.

If the identification of the colonies confirms the presence of the specified microorganism (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans or Escherichia coli*) the result is expressed as follows:

"Presence of the specified microorganism (*name of the species*) in the sample *S*."

If no growth after enrichment is observed and/or if the identification of the colonies does not confirm the presence of the specified microorganism (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans or Escherichia coli*), the result is expressed as follows:

"Absence of the specified microorganism (*name of the species*) in the sample, *S*."

3.4 Detection of specified and non-specified microorganisms

Two International Standards describe how to detect specified and non-specified microorganisms.

— The principle of ISO 18415 is to perform an enrichment by using a non-selective broth incubated under defined conditions to increase the number of microorganisms without the risk of inhibition by the selective ingredients that are present in selective/differential growth media followed by isolation on a non-selective medium incubated under defined conditions and identification conducted according to need by using appropriate incubation conditions and suitable identification tests.

For each species of specified microorganism, and if the identification of the colonies confirms the presence of this species, the result is expressed as follows:

"Presence of (*name of the species*) in the sample, *S*".

If growth is observed after enrichment and if the colonies are isolated and recognized as non-specified microorganisms, the result is expressed as follows:

"Presence of (*name of the species* or main morphological characteristics) in the sample, *S*, and absence of specified microorganisms".

If no growth after enrichment is observed, the result is expressed as follows:

"Absence of aerobic mesophilic bacteria and yeast (specified microorganisms included) in the sample, *S*".

— ISO 21149 describes a method for the detection of aerobic mesophilic bacteria.

The enrichment method described in ISO 21149 consists of incubation under defined conditions of a defined quantity of the sample (*S*) in a non-selective broth containing suitable neutralizers and/or dispersing agents followed by a transfer of a defined quantity of the previous suspension on non-selective solid agar medium under defined conditions. The difference between ISO 18415 and ISO 21149 is that the neutralization of the antimicrobial activity in ISO 21149 is only checked for bacteria, while ISO 18415 refers to both bacteria and yeast.

Therefore, if no growth after enrichment is observed, the results are expressed as follows:

"Absence of aerobic mesophilic bacteria (specified bacteria included) in the sample *S*".

If growth is observed after enrichment, the results are expressed as:

"Presence of aerobic mesophilic bacteria in the sample *S*".

NOTE Enrichment methods are not appropriate (temperature, culture media) to detect moulds. Therefore, even if no growth after enrichment is observed, it is necessary to look for moulds using appropriate culture conditions (see ISO 16212).

4 Antimicrobial preservation

4.1 General requirements

The antimicrobial protection of a cosmetic product (cosmetic formulation in its final container) can come from many sources, such as the following:

— chemical preservation;

— inherent characteristics of the formulation;

— package design;

— manufacturing process

When evaluating the overall antimicrobial protection of a cosmetic product, these different sources should be taken into account in a microbiological risk assessment.

ISO 11930 describes a procedure for the interpretation of data generated by the preservation efficacy test (if appropriate) and by the microbiological risk assessment

4.2 Evaluation of the preservation of a cosmetic formulation

A preservation efficacy test or challenge test is commonly used to evaluate the preservation of a cosmetic formulation. This test is not required for those cosmetic products for which the microbiological risk has been determined to be low (see ISO 29621).

This test is primarily designed for water-soluble or water-miscible cosmetic products and can require adaptation, for example, to test products in which water is the internal phase. The test described in ISO 11930 involves, for each test microorganism, placing the formulation in contact with a calibrated inoculum, and then measuring the changes in the microorganism count at set time intervals for a set period and at a set temperature.

As for the microbial content tests, the possible inhibition of microbial growth by the sample should be neutralized to allow the detection of viable microorganism. In all cases and whatever the methodology, the neutralization of the antimicrobial properties of the product should be checked and demonstrated.

The microorganism counts are converted in log reduction values and compared with 2 sets of criteria expressed as minimum log reduction.

— Criteria A, whereby the formulation is protected against microbial proliferation that may present a potential risk for the user and no additional factors are considered.

— Criteria B, whereby the level of protection is acceptable if the risk analysis demonstrates the existence of control factors not related to the formulation indicating that the microbiological risk is tolerable for the cosmetic product.

The inherent variability in microbial counts should be taken into consideration when comparing the obtained values and the preset criteria A or B. A deviation of 0,5 log units from the preset criteria is considered acceptable.

4.3 Evaluation of the preservation of a cosmetic product

The evaluation of the antimicrobial protection of a cosmetic product combines the following elements:

a) the characteristics of its formulation (see ISO 29621) or the results of the preservation efficacy test (if performed), or both;

b) the characteristics of the cosmetic product in conjunction with the production condition (see ISO 22716 and ISO 29621), the packaging type and, if justified, recommendations for use of the product (see ISO 29621).

5 Examples of microbial content results

5.1 General

Depending on the expected level of contamination of the sample to be tested, different approaches can be used (see Annex C).

5.2 Eye make-up remover

A water-based preserved product, however with a risk of contamination during manufacturing considered low, based on product history.

— Neutralization of the antimicrobial properties demonstrated to be suitable for a product dilution of 1/10.

— Enrichment in non-selective media according to ISO 18415 (1 ml sample): no detection.

Result: absence of aerobic mesophilic bacteria and yeast (specified microorganisms included) in 1 ml.

— Enumeration of yeast and mould according to ISO 16212: no recovery (1/10 dilution factor).

Result: <10 cfu of yeast and mould/ml.

The microbiological limit for products specifically intended for the eye area are as follows (see ISO 17516):

— total aerobic mesophilic microorganisms (bacteria plus yeast and mould) $<1 \times 10^2$ cfu per g or ml;

— absence of specified microorganisms in 1 g or ml.

Interpretation of results: → Product meets ISO 17516, provided that the microbiological risk is controlled according to ISO 11930 or ISO 29621 (no ability to proliferate in the product).

5.3 Mascara

A preserved oil in water emulsion with raw materials of natural origin (mineral pigments): likelihood of low-level of contamination (bacterial and fungal spores).

— Neutralization of the antimicrobial properties demonstrated to be suitable for a product dilution of 1/10.

— Enumeration of bacteria according to ISO 21149.

Count obtained for the 1/10 dilution: plate 1: 14 colonies, plate 2: 16 colonies, mean: 15 colonies.

Results: estimated number (see ISO 21149) 150 cfu of aerobic mesophilic bacteria/g.

— Enumeration of yeast and mould according to ISO 16212: no recovery (1/10 dilution factor).

Result: <10 cfu of yeast and mould/g.

— Detection of specified microorganisms according to ISO 18415 or according to ISO 21150, ISO 22717, ISO 22718 and ISO 18416 (1 g sample): no detection.

Result: absence of specified microorganisms in 1 g.

The microbiological limit for products specifically intended for the eye area are as follows (see ISO 17516):

— total aerobic mesophilic microorganisms (bacteria plus yeast and mould) $<1 \times 10^2$ cfu per g or ml;

— absence of specified microorganisms in 1g or ml.

The interpretation of results are as follows:

— total aerobic mesophilic microorganisms (bacteria plus yeast and mould): 150 cfu/g;

— due to inherent variability of the plate count method (see ISO 17516), result is considered out of limit if >200 cfu/g or ml;

— absence of specified microorganisms in 1 g or ml.

→ Product meets ISO 17516, provided that the microbiological risk is controlled according to ISO 11930 or ISO 29621 (no ability to proliferate in the product).

5.4 Face cream

A water-based preserved product with no product history.

— Neutralization of the antimicrobial properties demonstrated to be suitable for a product dilution of 1/10.

— Enumeration of bacteria according to ISO 21149 for 1/10 dilution: no recovery.

Result: <10 cfu of bacteria/g.

— Enumeration of yeast and mould according to ISO 16212 for the 1/10 dilution (1 g sample): no recovery (1/10 dilution factor).

Result: <10 cfu of yeast and mould/g.

— Detection of specified microorganisms according to ISO 18415 or according to ISO 21150, ISO 22717, ISO 22718 and ISO 18416: detection of *Pseudomonas aeruginosa*.

Result: presence of *Pseudomonas aeruginosa* in 1 g.

Microbiological limit for products non-specifically intended for under three year old children and eye area or mucous membranes are as follows (see ISO 17516):

— total aerobic mesophilic microorganisms (bacteria plus yeast and mould) $<1 \times 10^3$ cfu per g or ml;

— absence of specified microorganisms in 1g or ml.

Interpretation of results are as follows:

— total aerobic mesophilic microorganisms (bacteria plus yeast and mould): <10 cfu/g;

— presence of *Pseudomonas aeruginosa* in 1 g.

→ Product does not meet ISO 17516.

5.5 Shampoo

A water-based preserved product with no product history.

— Neutralization of the antimicrobial properties demonstrated to be suitable for a product dilution of 1/100 for bacteria and 1/10 for yeast and mould.

— Enumeration of bacteria according to ISO 21149:

  Count obtained for the 1/100 dilution: plate 1: 280 colonies, plate 2: 300 colonies, mean: 290 colonies.

  Result: $2,9 \times 10^4$ cfu of aerobic mesophilic bacteria/g.

— Enumeration of yeast and moulds according to ISO 16212 for the 1/10 dilution (1 g sample): no recovery (1/10 dilution factor).

  Result: <10 cfu of yeast and mould/g.

— Detection of specified microorganisms according to ISO 18415 or according to ISO 18416, ISO 21150, ISO 22717 and ISO 22718. Enrichment conducted with a dilution factor of 1/100 (1 g of sample into 100 ml of diluent): no detection.

  Result: absence of specified microorganisms in 1 g.

Microbiological limit for products non-specifically intended for under three year old children and eye area or mucous membranes (see ISO 17516):

— total aerobic mesophilic microorganisms (bacteria plus yeast and mould) $<1 \times 10^3$ cfu per g or ml

— absence of specified microorganisms in 1 g or ml.

Interpretation of results:

— total aerobic mesophilic microorganisms (bacteria plus yeast and mould): $2,9 \times 10^4$ cfu/g.

— due to inherent variability of the plate count method (see ISO 17516), result is considered out of limit if >2 000 cfu/g or ml

→ Product does not meet ISO 17516.

Manufacturers should follow an Out-of-Specification (OOS) procedure to confirm or reject the OOS results.

6 Examples of interpretation of preservation efficacy test results

Antimicrobial protection is based on a combination of formulation characteristics, production conditions and final packaging. The overall evaluation takes into account the microbiological risk assessment together with the preservation efficacy test results, if relevant. The evaluation criteria for the preservation efficacy test performed on the formulation are given in ISO 11930.

Table 1 — Evaluation criteria

log reduction values ($R_x = lgN_0\text{-}lgN_x$) required[a]								
Microorganisms	**Bacteria**			***C .albicans***			***A. brasiliensis***	
Sampling time	T7	T14	T28	T7	T14	T28	T14	T28
Criteria A	≥3	≥3 and NI[b]	≥3 and NI	≥1	≥1 and NI	≥1 and NI	≥0[c]	≥1
Criteria B	Not performed	≥3	≥3 and NI	Not performed	≥1	≥1 and NI	≥0	≥0 and NI

a In this test, an acceptable range of deviation of 0,5 log is accepted (see ISO 11930).

b NI: no increase in the count from the previous contact time.

c $R_x = 0$ when $\lg N_0 = \lg N_x$ (no increase from the initial count).

Table 2 — Examples of preservation efficacy tests performed according ISO 11930 on various cosmetic formulations with conclusion on the corresponding products

Examples	Formulation										Product	
	Challenge test results (log reduction values)								**Comments**	**Conclusion on the formulation**	**Existence of control factors not related to the formulation?**	**Conclusion on the antimicrobial protection of the product**
	Bacteria[a]			*C.albicans*			*A.brasiliensis*					
	T7	T14	T28	T7	T14	T28	T14	T28				
1	3,1	3,4	3,8	1,0	1,1	1,4	0,0	1,0		Pass for criteria A	Not required	The product is protected against microbial proliferation that may present a potential risk for the user.
2	**2,6**	3,4	3,8	**0,6**	1,1	1,4	**-0,3**	1,0	Deviation of 0,5 log is acceptable (see ISO 11930)	Pass for criteria A	Not required	The product is protected against microbial proliferation that may present a potential risk for the user.
3		3,1	3,5		1,1	1,3	0,1	0,5		Pass for criteria B	Yes (e.g: package is a pump)	The product complies with ISO 11930 on the basis of criterion B plus additional characteristics indicating that the microbial risk is tolerable.
4		3,1	3,8		1,0	1,1	**-0,4**	**-0,1**	Deviation of 0,5 log is acceptable (see ISO 11930)	Pass for criteria B	No	The product does not meet the requirements of ISO 11930.
5		**1,6**	**2,3**		1,1	1,5	0,2	1,6		Fail criteria A and B	Yes (strengthened control factors, e.g: single-dose plus aseptic filling)	The product is considered a tolerable microbiological risk provided that the microbiological quality of the finished product is ensured at the time of release.
6		**1,6**	**2,3**		1,1	1,5	0,2	1,6		Fail criteria A and B	No	The product does not meet the requirements of ISO 11930.

[a] Among *E. coli, P. aeruginosa* and *S. aureus*, select the strain with the lowest log reduction value.

Annex A
(informative)

Relationship between the ISO cosmetic microbiology standards

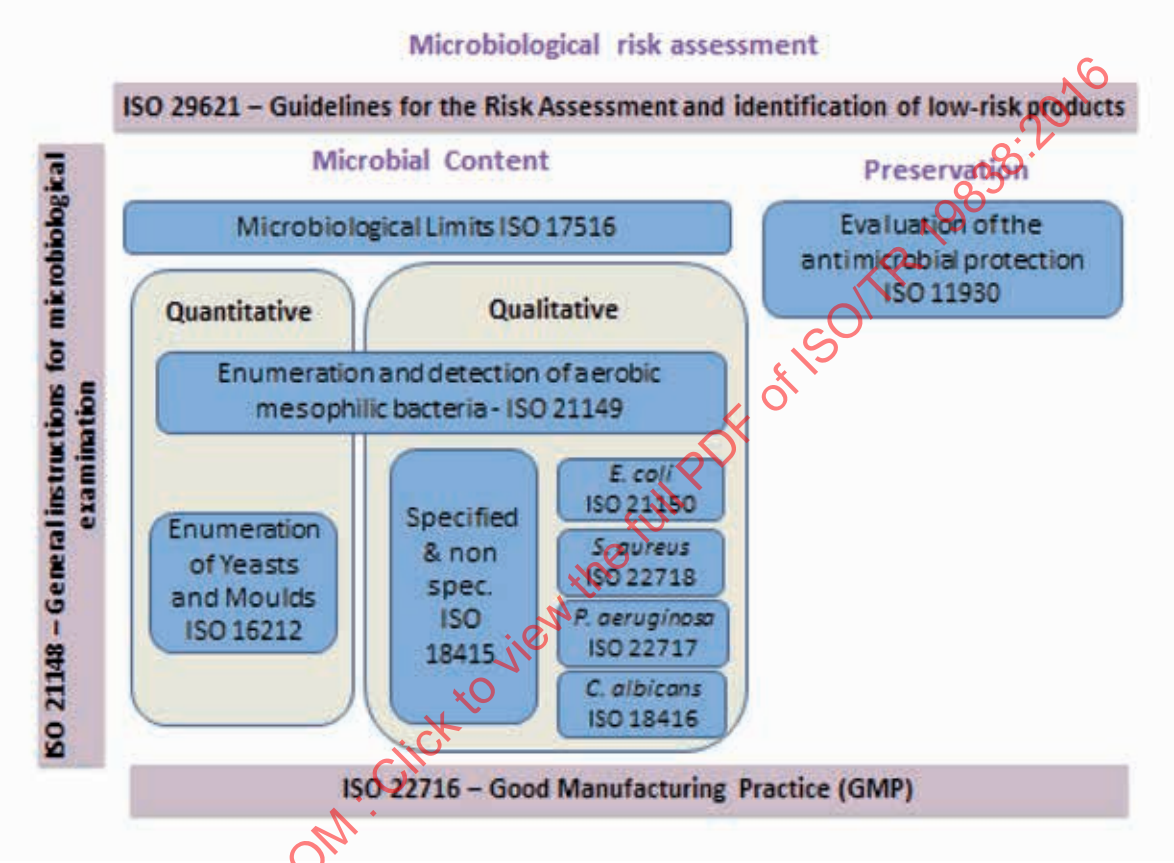

Figure A.1 — Microbiological risk assessment