
**Cosmetics — Microbiology —
Guidelines for the risk assessment
and identification of microbiologically
low-risk products**

*Cosmétiques — Microbiologie — Lignes directrices pour
l'appréciation du risque et l'identification de produits à faible risque
microbiologique*

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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

For an explanation on the voluntary nature of ISO 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 ISO/TC 217, *Cosmetics*.

This second edition cancels and replaces the first edition (ISO 29621:2010), which has been technically revised.

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. The second is to ensure that microorganisms introduced during normal product use will not adversely affect the quality or safety of the product.

The first step would be to perform a microbiological risk assessment of the product to determine if the cosmetic microbiological International Standards apply.

Microbiological risk assessment is based on a number of factors generally accepted as important in evaluating the adverse effects on product quality and consumer health. It is intended as a guide in determining what level of testing, if any, is necessary to assure the quality of the product. Conducting a microbiological risk assessment involves professional judgment and/or a microbiological analysis, if necessary, to determine the level of risk.

The nature and frequency of testing vary according to the product. The significance of microorganisms in non-sterile cosmetic products is to be evaluated in terms of the use of the product, the nature of the product and the potential harm to the user.

The degree of risk depends on the ability of a product to support the growth of microorganisms and on the probability that those microorganisms can cause harm to the user. Many cosmetic products provide optimum conditions for microbial growth, including water, nutrients, pH and other growth factors. In addition, the ambient temperatures and relative humidity at which many cosmetic products are manufactured, stored and used by consumers, will promote growth of mesophiles that could cause harm to users or cause degradation of the product. For these types of products, the quality of the finished goods is controlled by applying cosmetic good manufacturing practices (GMPs) (see ISO 22716) during the manufacturing process, using preservatives and conducting control tests using appropriate methods.

The likelihood of microbiological contamination for some cosmetic products is extremely low (or non-existent) due to product characteristics that create a hostile environment for survival/growth of microorganisms. These characteristics are elaborated in this document. While the hazard (adverse effects on product quality and consumer health) may remain the same for these products, the likelihood of an occurrence is extremely low. These products identified as “hostile” and produced in compliance with GMPs pose a very low overall risk to the user.

Therefore, products that comply with the characteristics outlined in this document do not require microbiological testing.

This document gives guidance to cosmetic manufacturers and regulatory bodies to determine when, based on a “risk assessment,” the application of the microbiological International Standards for cosmetics and other relevant methods is not necessary.

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Cosmetics — Microbiology — Guidelines for the risk assessment and identification of microbiologically low-risk products

1 Scope

This document gives guidance to cosmetic manufacturers and regulatory bodies to help define those finished products that, based on a risk assessment, present a low risk of microbial contamination during production and/or intended use, and therefore, do not require the application of microbiological International Standards for cosmetics.

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 risk

effect of uncertainty on objectives

Note 1 to entry: Microbiological risk is associated with the ability of a product to

- support the growth of microorganisms and the probability that those microorganisms can cause harm to the user;
- support the presence of specified microorganisms as identified in cosmetic microbiological International Standards, e.g. ISO 18415, ISO 18416, ISO 22717, ISO 22718 and ISO 21150.

[SOURCE: ISO Guide 73:2009, 1.1, modified]

3.2 risk assessment

overall process of risk identification, *risk analysis* (3.3) and *risk evaluation* (3.4)

[SOURCE: ISO Guide 73:2009, 3.4.1]

3.3 risk analysis

process to comprehend the nature of *risk* (3.1) and to determine the level of risk

[SOURCE: ISO Guide 73:2009, 3.6.1]

3.4 risk evaluation

process of comparing the results of *risk analysis* (3.3) with *risk criteria* (3.5) to determine whether the *risk* (3.1) and/or its magnitude is acceptable or tolerable

[SOURCE: ISO Guide 73:2009, 3.7.1]

3.5 risk criteria

term of reference against which the significance of a *risk* (3.1) is evaluated

[SOURCE: ISO Guide 73:2009, 3.3.1.3, modified]

3.6 microbiologically low-risk product

product whose environment denies microorganisms the physical and chemical requirements for growth and/or survival

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

4 Risk assessment factors

4.1 General

A number of product characteristics needs to be evaluated when performing a microbial risk assessment to determine if that product should be subjected to the published microbiological International Standards for cosmetics or other relevant methods. These characteristics include the composition of the product, the production conditions, packaging and a combination of these factors.

4.2 Composition of the product

4.2.1 General characteristics

Products with certain physico-chemical characteristics do not allow the proliferation of microorganisms of concern to cosmetic products. Any number of physico-chemical factors or combinations thereof in a product can create a hostile environment that will not support microbial growth and/or survival. Combinations of sub-lethal factors will increase the hostility of the environment and increase the lag phase. If the environment is hostile enough, the lag phase will be extended to infinity and therefore cause cell death. Combinations of lethal factors will cause rapid cell death. The following factors should be considered in determining whether cosmetic products present a hostile environment.

4.2.2 Water activity, a_w , of formulation

Water is one of the most important factors controlling the rate of growth of an organism. It is not the total moisture content that determines the potential for growth but the available water in the formulation. The metabolism and reproduction of microorganisms require the presence of water in an available form. The most useful measurement of water availability in a product formulation is water

activity, a_w . Water activity is defined as the ratio of the water vapour pressure of the product to that of pure water at the same temperature [see [Formula \(1\)](#)]:

$$a_w = \frac{p}{p_0} = \frac{n_2}{(n_1 + n_2)} \quad (1)$$

where

p is the vapour pressure of the solution;

p_0 is the vapour pressure of pure water;

n_1 is the number of moles of solute;

n_2 is the number of moles of water.

When a solution becomes more concentrated, vapour pressure decreases, and the water activity falls from a maximum of 1,00 (a_w for pure water). These conditions have been categorized with respect to their capacity to grow and produce metabolites in various conditions and values of a_w . The influence of reduced a_w on microorganisms is well documented. As the amount of free water in a formulation is reduced (decrease in a_w), the microorganism is faced with the challenge of maintaining a state of turgor within the cell. Loss of turgor will result in slower growth and eventually death of the cell. Many organisms survive under conditions of low a_w but will not grow. Lowered a_w causes an increase in the lag phase of growth, decrease in growth and decrease in total cell count. At very low values of a_w , it can be assumed that the lag phase becomes infinite, i.e. no growth. In low a_w environments, cells shall use energy to accumulate compatible solutes to maintain internal pressure. The growth of most bacteria is confined to an a_w above 0,90. Some yeast and mould can grow at a much lower a_w with a limiting value above 0,60 (see References [1] and [2]).

Listed in [Table 1](#) are examples of the minimum water activity levels required for growth of selected microorganisms.

Table 1 — Approximate minimum water activity (a_w) required for growth of selected microorganisms

Bacteria	Water activity (a_w)	Molds and yeast	Water activity (a_w)
<i>Pseudomonas aeruginosa</i>	0,97	<i>Rhizopus nigricans</i>	0,93
<i>Bacillus cereus</i>	0,95	<i>Mucor plumbeus</i>	0,92
<i>Clostridium botulinum</i> , Type A	0,95	<i>Rhodotorula mucilaginosa</i>	0,92
<i>Escherichia coli</i>	0,95	<i>Saccharomyces cerevisiae</i>	0,90
<i>Clostridium perfringens</i>	0,95	<i>Paecilomyces variotii</i>	0,84
<i>Lactobacillus viridescens</i>	0,95	<i>Penicillium chrysogenum</i>	0,83
<i>Salmonella</i> spp.	0,95	<i>Aspergillus fumigatus</i>	0,82
<i>Enterobacter aerogenes</i>	0,94	<i>Penicillium glabrum</i>	0,81
<i>Bacillus subtilis</i>	0,90	<i>Aspergillus flavus</i>	0,78
<i>Micrococcus lysodeikticus</i>	0,93	<i>Aspergillus brasiliensis</i>	0,77
<i>Staphylococcus aureus</i> (see Reference [2])	0,86	<i>Zygosaccharomyces rouxii</i> (osmophilic yeast)	0,62
<i>Halobacterium halobium</i> (halophilic bacterium)	0,75	<i>Xeromyces bisporus</i> (xerophilic fungi)	0,61

The water activity values in [Table 1](#) should be considered as reference points, since microbial growth may occur at lower values depending on differences in temperature, pH or nutrient content of the product formulation. Even though water activity values are important in assisting in the

risk analysis for microbial contamination, water activity should not be used as the sole indicator in determining whether product testing is necessary for a particular product formulation. USP indicates that pharmaceutical products with water activities below 0,75 prevent microbial growth. Generally, anhydrous product formulations will have low water activity levels (e.g. <0,7) (see References [3], [4] and [5]). A water activity level greater than 0,8 is required for microorganisms to proliferate in a product formulation (see References [6] and [7]). Because the possibility of microbial proliferation is non-existent in product formulations that have a water activity level lower than 0,7, there is no need to conduct preservative challenge testing in these types of product formulations. In the absence of chemical preservatives, a low water activity level alone is more than sufficient to keep a product adequately preserved (see Reference [8]). Similar values may apply to cosmetics. Other factors, such as manufacturing and filling temperatures, should be taken into consideration to determine if a product requires further microbiological testing.

4.2.3 pH of formulation

The use of acidic pH is a common practice in the food industry for protection against bacteria and these same principles apply to cosmetics. The combination of acidic pH and a_w has been thoroughly studied (see Reference [9]). In many instances, the level of inhibition on microbial activity depends on the specific acid being used. Acidic conditions around pH 5 favour mould and yeast proliferation but will not support bacterial growth. As the pH falls below pH 3,0, the conditions for growth of yeast become hostile (see Reference [10]); this is because intracellular pH has to be maintained within relatively narrow limits.

Alkaline pH may also create a hostile environment and may in some products be used as part of their preservative system. Liquid soaps with alkaline pH (pH 9,0 to pH 10,0) present an environment unfavourable for the growth of some microorganisms (see Reference [11]). Hair curl relaxers, due to their extreme pH (around 12), prevent the growth of virtually all microorganisms that would be likely to contaminate cosmetic products (see Reference [12]).

The reason for this is that the extreme pH, either acidic or alkaline, makes it necessary for microorganisms to expend energy on maintenance of intracellular pH rather than growth. When pH is used in combination with chelating agents, glycols, antioxidants, water activity and high surfactant levels, an environment can be created which will not support microbial growth.

These concepts may be visualized as “hurdles” that microorganisms shall overcome in order to grow (see Reference [13]).

In certain product types, where extreme pH levels are reported, those considered pH $\leq 3,0$ and pH $\geq 10,0$ do not require microbiological testing, including both challenge-test and end product testing. At all other pH values (>3,0 but <10,0), a combination of pH and other physico-chemical factors needs to be evaluated to determine potential risk. Data to support the conclusion that the microbiological risk is low may need to be generated, either through experimental design or review of product history.

4.2.4 Raw materials that can create a hostile environment

4.2.4.1 Alcohol

Microbial growth is prevented in aqueous systems containing ≥ 20 % by volume mass of absolute ethyl alcohol. However, lower alcohol levels (5 % to 10 %) may have additive or synergistic activity when combined with other physico-chemical factors (see Reference [14]).

Ethanol, *n*-propanol and *iso*-propanol are the most frequently used aliphatic alcohols in cosmetic preparations (see Reference [15]). Their antimicrobial efficacy increases with molecular weight and chain length. The concentration in which they are present in a product determines whether they will kill or merely inhibit microorganisms. Data in the literature indicate that the microbiostatic effect of alcohol is quite high in the range of 10 % to 20 %, and will allow for a reduction in preservation. Depending on the pH of the substrate, 15 % to 18 % ethyl alcohol has generally been considered acceptable for preservation (see Reference [16]).

Products containing alcohol levels ≥ 20 % by volume mass do not require microbiological testing (challenge-test and end product testing). At levels below 20 %, other physico-chemical factors need to be evaluated to determine potential risk. Data to support the conclusion that the microbiological risk is low may need to be generated, either through experimental design or review of product history.

4.2.4.2 Ammonia and monoethanolamine

Ammonia and monoethanolamine, two alkaline agents, are commonly used in hair dyes where they serve three important purposes: i) swell the hair fibre to allow dye precursors to better penetrate, ii) generate the active peroxide species necessary for melanin bleaching and dye formation, iii) participate to the bleaching of melanin.^[12] They are also used in waving lotions, which involve the reduction of the structural disulphide bonds of the hair. They facilitate the penetration of waving lotion, which is usually alkaline and is applied to the hair once it is set in rollers. Besides these primary functions, as alkalizers, ammonia and monoethanolamine are expected to create a hostile environment for microbial growth in the products in which they are used (see References [17] and [18]).

Products containing ammonia level $\geq 0,5$ % and/or monoethanolamine level ≥ 1 % deny microorganisms the physical and chemical requirements for growth and/or survival, and can therefore be considered as microbiologically low-risk (see Reference [15]).

4.2.4.3 Polar organic solvents (e.g. ethyl acetate and butyl acetate)

Butyl acetate and ethyl acetate are organic solvents commonly used in nail polishes. These are basically made from nitrocellulose dissolved in solvents. Solvents are liquids used to mix the other ingredients (film formers, resins, plasticizers, pigments, etc.) in a nail polish to yield a uniformly spread product.

Besides this primary function, these organic solvents, when used at concentration >10 %, create a hostile environment for microbial growth in the formulae in which they are used (see Table 2).

Mixtures of these solvents, which are characteristic of nail varnish compositions, have a high microbiocidal activity on the tested strains within a short time (see Reference [19]).

Solvent-based nail polishes can therefore be considered low microbiological risk and do not require microbiological testing (challenge-test and end product testing).

4.2.4.4 Other raw materials that can create a hostile environment

The use of certain raw materials in cosmetic formulations will help to create an environment that is hostile to microbial growth. Data to support the conclusion that microbial growth has been inhibited may need to be generated, either through literature reference, experimental design or review of product history. The following are examples of some materials that create such an environment.

- a) Strong oxidizing agents (e.g. hydrogen peroxide) (see Reference [20]) or strong reducing agents (e.g. thiol compounds). Hydrogen peroxide has been shown to possess a wide spectrum of antimicrobial activity, in that it is active against bacteria, yeasts, fungi, viruses and spores. Most strains show a complete inhibition with 3 % hydrogen peroxide (see Reference [21]).
- b) Oxidizing dyes.
- c) Aluminium chlorohydrate and related salts.

The use of high levels of aluminium chlorohydrate (w/w 25 %) in certain deodorants and antiperspirants gives rise to an acidic pH and a low a_w value, making these products intrinsically hostile to microbial growth (see Reference [21]). In these conditions, the microbiological risk can be considered to be controlled and these products do not require microbiological testing (challenge-test and end product testing).

d) Propellant gases.

In the case of cosmetics where a propellant gas (e.g. dimethyl ether, isobutane) is used to help deliver the product (hairsprays, deodorants, shaving foam, etc.), microbial growth is hindered by the fall in the partial pressure of oxygen, and in certain cases by the intrinsic inhibiting effect of the propellant gas (see References [3], [22], [23], [24] and [25]).

e) Other substances.

Other raw materials can be hostile to microbial growth. Data to support the conclusion that the microbiological risk is low may need to be generated, either through literature reference, experimental design or review of product history.

4.3 Production conditions

Certain aspects of the manufacturing and filling processes (e.g. high temperature) may reduce the microbiological risk to a cosmetic product. As with pH, there is an optimum temperature range for microbial growth. Low temperatures will allow for slow growth and raising temperatures could potentially increase growth. As the temperature rises above optimum, growth is inhibited and microorganisms are killed. Heat is used to control microorganisms either by applying a temperature adequate for rapid kill or by maintaining a temperature above optimum for an extended period of time (see Reference [26]).

A temperature above 65 °C can cause thermal inactivation of the microbial bio-burden in a product formulation. With a 10 min hold time at a temperature of 65 °C, most vegetative bacterial cells die due to degradation of cellular proteins.

Based on the above information, microbial content testing on product formulations that are filled at a temperature above 65 °C is not required. Periodic testing of the product or verification of the lethality of the process temperature should be considered. It is also recommended that periodic review of manufacturing and filling be performed to ensure there have been no changes to the conditions of the process.

4.4 Packaging

The type of packaging components chosen for the presentation of a cosmetic product has a direct influence on the risk of its contamination in use (see Reference [27]) and shall be taken into account in the microbiological risk evaluation during use.

- Certain packaging components give physical protection against contamination from consumer use (e.g. a pump dispenser, single dose units) and contribute to the protection and preservation of a formulation.
- Other factors such as a small product volume limiting the number of uses or an indication of short duration of use also contribute to the protection of formulation.
- Certain presentations, e.g. pressurized delivery or unit-dose, provide full protection of the cosmetic formulation from contamination during use. If the product is microbiologically acceptable when marketed, it will remain so throughout its use. In this case, the microbiological risk during use is low, based on the high level of protection provided by the package.

4.5 Combined factors

Combinations of the factors mentioned in this document can create an environment that is hostile to microbial growth or survival. These combined factors should be taken into account when determining if a product is subject to the appropriate microbiological standards regarding testing and/or product stability (see Reference [28]).

The exemption from testing should be based on appropriate justification. This determination is the responsibility of the manufacturer. Data to support the conclusion that the microbiological risk is

low may need to be generated through literature reference, experimental design or review of product history.

5 Identified low-risk products

After review of 4.1 to 4.5, products that meet any of the following product characteristics and their combinations may be considered as examples of low-risk products.

Table 2 — Examples of low-risk products

Physico-chemical factor	Limit	Example
pH	$\leq 3,0$	Skin peels (glycolic acid)
pH	$\geq 10,0$	Hair relaxers
Anhydrous	—	Body oil, pencils
Ethanol or other alcohol	$\geq 20 \%$	Hair sprays, tonics, perfumes
Filling temperature	$\geq 65,0 \text{ }^\circ\text{C}$	Lip balms, lipsticks, cream blushes
Water activity (a_w)	$\leq 0,75^a$	
Organic solvents: Ethyl acetate Butyl acetate	$>10 \%$ $>10 \%$	Solvent-based products: e.g. nail enamels
Alkaline compounds: Ammonia Monoethanolamine	$\geq 0,5 \%$ $\geq 1 \%$	Oxidizing products: e.g. hair dyes, perms
Aluminium chlorohydrate and related salts	$\geq 25 \%$	Antiperspirants
Hydrogen peroxide	$\geq 3 \%$	Hair lightening, bleaching, perms
NOTE Soap bars, syndets and solid cleansing bars are considered low risk because of low water activity and high pH.		
^a See Reference [29].		

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