As a result of their composition and the substances used, a large majority of cosmetics is at risk from microbial contamination. Creams, lotions, products containing surfactants and other aqueous products more or less provide the ideal living conditions for a variety of microorganisms. The microbiological condition of cosmetics must therefore be such that there is no potential risk for the consumer or user at any time during the entire life cycle of the product’s use. Article 3 of Regulation (EC) No 1223/2009 (EU Cosmetics Regulation) refers to products which must be safe for human health when used under normal or reasonably foreseeable conditions of use. This requirement includes the microbiological safety and harmlessness of cosmetics.

Regulation (EC) No 1223/2009 (Annex I, Part A) also deals with the microbiological quality of products as part of the safety report which must be compiled for each product placed on the market. It lists the microbiological quality requirements as follows: “Microbiological quality: The microbiological specifications of the substance or mixture and the cosmetic product. Particular attention shall be paid to cosmetics used around the eyes, on mucous membranes in general, on damaged skin, on children under three years of age, on elderly people and persons showing compromised immune responses.”

Furthermore results of a preservation challenge test are required (see below). Further requirements and recommendations can be found in the “SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation” 2. Herein routine microbiological testing and analysis of each batch of the finished cosmetic product is demanded and quantitative as well as qualitative limits are specified (see below and Table 2). ISO 17516 3 indicates that production must take place under hygienic conditions (see comprehensive requirements of ISO 22716 [Cosmetics – Good Manufacturing Practices4]). Although it does not demand sterile products, the products may neither contain excessive quantities of microorganisms nor specific microorganisms which have the potential to compromise the quality of the product.

Above requirements result in the fact that manufacturers of cosmetics must ensure the microbiological harmlessness of their products with regard to health protection and safety. This harmlessness must be part of a suitable microbiological quality management (MQM) system 5. In addition to the testing of finished products mentioned above, this MQM strategy comprises the testing of raw materials, semi-finished products and workplace hygiene checks. The development phase (upscaling) and the implementation of a safe, reproducible production process (good manufacturing practice; GMP) must also be included in this process.

The manufacturer or responsible person is allowed a relatively high level of freedom to select the methods, type and extent of product testing. Ultimately, it must be ensured that cosmetics do not contain any microorganisms, metabolic products or resulting toxins which could damage human health (pathogenicity).

Basics of microbiological testing procedures

It is recommended that only published and validated testing methods are used for the microbiological testing of finished cosmetic products as well as corresponding raw materials (including the water used). Naturally the (additional) use of “in-house methods” or rapid methods is also possible on the condition that these methods are sufficiently validated and offer the same analytical reliability and precision as the previously described procedures. Regular participation in interlaboratory microbiological tests provides valuable information regarding the quality and operation method of the corresponding microbiological testing laboratory. General guidance on the microbiological testing of cosmetics, including on the analysis equipment used, lab hygiene, manufactur-
ing culture media and reagents as well as generally dealing with lab samples can be found in ISO 21148. The microbiological testing of cosmetics is generally carried out using cultural methods with the corresponding standard media and additional enrichment media. The corresponding testing standards define parameters such as culture media, sample quantity tested, diluting solution, etc.

As cosmetics contain substances with an antimicrobial effect in most cases, it is always necessary to ensure sufficient neutralisation in all microbiological tests in order to prevent false negative results.

Here a basic distinction has to be made between testing the microbiological status of a product or raw material and testing for sufficient preservation (preservation challenge test). Please note, the following test organisms are just exemplary. The actual multitude of potential microorganisms in cosmetic products must always be taken into account; a modification or amendment to corresponding testing procedures is advisable if needed.

### Testing microbiological status

During the testing of a product's microbial status, both quantitative (indication of results: number of microorganisms per product quantity tested) and qualitative procedures (indication of results: microorganisms present or absent in a certain product quantity) are used. In products with unremarkable microbiological findings, the result is generally <10 CFU/g or microbe(s) absent in 1g of product (CFU / g = colony-forming units per gramme of product). If any microorganisms are detected in the finished product, control mechanisms must be determined within the scope of the MQM system. This includes the determination of the period after opening (PAO = Period after opening) and represents the standard method of testing microbiological stability.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Designation</th>
<th>Measurement principle</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISO 21149</td>
<td>Aerobic mesophilic bacteria (yeasts, moulds also detectable)</td>
<td>Quantitative: Decimal dilution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Qualitative: Enrichment in non-selective liquid medium, streak on non-selective culture media</td>
</tr>
<tr>
<td>ISO 18415</td>
<td>Specified and non-specified microorganisms</td>
<td>Qualitative: Enrichment in non-selective liquid medium, streak on non-selective culture media Specified microorganisms: Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Candida albicans Non-specified microorganisms: all other microorganisms</td>
</tr>
<tr>
<td>ISO 16212</td>
<td>Yeasts and moulds</td>
<td>Quantitative: Decimal dilution</td>
</tr>
<tr>
<td>ISO 18416</td>
<td>Candida albicans</td>
<td>Qualitative: Enrichment in non-selective liquid medium; streak on selective culture media</td>
</tr>
<tr>
<td>ISO 21150</td>
<td>Escherichia coli</td>
<td>Qualitative: Enrichment in non-selective liquid medium; streak on selective culture media</td>
</tr>
<tr>
<td>ISO 22717</td>
<td>Pseudomonas aeruginosa</td>
<td>Qualitative: Enrichment in non-selective liquid medium; streak on selective culture media</td>
</tr>
<tr>
<td>ISO 22718</td>
<td>Staphylococcus aureus</td>
<td>Qualitative: Enrichment in non-selective liquid medium; streak on selective culture media</td>
</tr>
</tbody>
</table>

Table 1: Methods for colony counting and testing specific microorganisms.

### Testing for efficacy of antimicrobial preservation (Preservation challenge test)

An appropriate preservation system is necessary for all cosmetic products which are subject to the risk of microbial growth so that stability and consequently the safety of the product can be ensured.

Experimentally, this test of microbiological product stability is carried out with the help of a preservation challenge test (abbr. PCT). This is used synonymously with the terms "testing for efficacy of antimicrobial preservation" and "assessment of antimicrobial protection".

The test serves to prove that a preservation system is effective and represents the standard method of testing microbiological stability and durability during the product's period of storage and use. As clarified above, the results of this test procedure are a mandatory component of the safety report. Along with other data (analytical values regarding preservatives, data on physical stability, packaging, way of use, experience with similar formulations, etc.), the results of a PCT are an important component for determining the period after opening (PAO = Period after opening).14

The PCT is generally carried out in accordance with ISO 11930 methodology. Usually this test is not necessary in case of products for which the microbiological risk has been determined as...
low (see chapter: “Microbiological risk assessment” chapter). During testing, each cosmetic product is inoculated with a certain test organism and the change in number of this microorganism measured at certain intervals over a certain period of time. The determination of the logarithmic ratio of inoculum (respective test microbe) to microbial count after certain periods of time (up to 28 days) results in characteristic degradation rates which are then evaluated and allow an assessment of microbiological stability. The following five microorganisms are used in this test in accordance with ISO 11930 (Figure 1):

Microbiological risk assessment

The microbiological testing of cosmetics should always be preceded by a corresponding risk analysis of the respective product. During the risk assessment, the following general factors are especially important:

- Location of product use (skin, hair, eye, mucous membranes, damaged areas of skin)
- Users (adults, children, children under the age of 3, people with weakened immune systems)

It should be noted that the microorganisms named here may only be viewed as a guide and that, depending on the raw materials used and taking into consideration the production process, it may be wise and advisable to test for further microbes. Furthermore, the results of a microbiological test must always be viewed within the overall context of a product (product composition, way of use, packaging, etc.), as all these factors may promote or slow down microbial growth.

Microbiological standard and limit values

From a purely legal perspective, there are currently no limit values for the microbiological contamination of cosmetics, but there is a range of standard values regarding total microbial count and the exclusion of specified microorganisms published in the recommendations of various associations and pharmacopoeia (see Table 2). Ultimately, it is down to the manufacturer or responsible person to decide whether a product may be categorised as marketable based on a potential microbial contamination.

<table>
<thead>
<tr>
<th>Microbial count (CFU/g or CFU/ml)</th>
<th>Specified microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISO 17516 ³</td>
<td>Children under the age of 3, products used in the eye area, mucous membranes: ≤100 Other products: ≤1000</td>
</tr>
<tr>
<td>SCCS *²</td>
<td>Children under the age of 3, eye area, mucous membranes: ≤100 (Category 1) Other products (Category 2): ≤1000</td>
</tr>
<tr>
<td>Pharmacopoeia ¹⁶–¹⁸</td>
<td>Products for topical application: TAMC: ≤100 TYMC: ≤10 Products with cutaneous use: absent per 1g or ml: Pa, Sa, Ca, Ec</td>
</tr>
<tr>
<td>Cosmetics Europe ¹⁹</td>
<td>Newborns, products in the eye area: ≤100 Other products: ≤1000 absent per 0.1g or ml: Pa, Sa, Ca</td>
</tr>
</tbody>
</table>

Table 2: Microbiological requirements of cosmetics.
* Reference to ISO 17516
Pa = Pseudomonas aeruginosa; Sa = Staphylococcus aureus; Ca = Candida albicans; Ec = Escherichia coli; TAMC = Total Aerobic Microbial Count; TYMC = Total Combined Yeast and Mould Count
• Potential pathogenicity of microbes detected
• Potential changes of the product during the course of its use

Along with these general factors, the risk of microbial contamination always depends on the individual product characteristics too (properties and composition of a product). Production conditions, packaging characteristics and possible recommendations for the product’s use also have an influence. 20, 21.

While some products offer the ideal conditions for potential growth regarding nutrients, water content, pH value and other factors, the microbial risk of other products is significantly reduced or effectively ruled out. The following three product categories may be distinguished based on their product characteristics (see also: ISO 29621) 21:

– Products with a low risk of microbial growth (exemplary):
  • Alcohol content ≥ 20 %
  • pH value ≤3 and ≥ 10
  • Water activity ≤ 0.75
  • Filling temperature ≥ 65°C
  Normally, routine microbiological testing of such products is not necessary (colony counting and PCT).

– Single-use products and products which cannot be opened:
  Generally, only the microbial status of these products must be considered on a routine basis.

– All other products:
  Generally, both a PCT and a determination of the microbial status are necessary for this product category.

It should also be mentioned that the assessment of a potential microbial risk for a certain cosmetic product always represents a case by case decision. The ISO 29621 provides valuable help here. 21.

**Literature**

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5) Leitfaden für Mikrobiologisches Qualitätsmanagement (MQM) kosmetischer Mittel [Guidelines for Microbiological Quality Management (MGM) Cosmetics], IKW [The German Cosmetic, Toiletry, Perfumery and Detergent Association], April 1998
6) ISO 21148, Cosmetics – Microbiology – General instructions for microbiological examination, October 2005
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8) ISO 18415, Cosmetics – Microbiology – Detection of specified and non-specified microorganisms, September 2007
9) ISO 16212, Cosmetics – Microbiology – Enumeration of yeast and mould, October 2008
10) ISO 18416, Cosmetics – Microbiology – Detection of Candida albicans, December 2015
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12) ISO 22717, Cosmetics – Microbiology – Detection of Pseudomonas aeruginosa, November 2015
13) ISO 22718, Cosmetics – Microbiology – Detection of Staphylococcus aureus, December 2015
15) ISO 11930, Cosmetics – Microbiology – Evaluation of the antimicrobial protection of a cosmetic product, April 2012
16) European Pharmacopeia (Ph. Eur.) 8.0, 5.1.4 Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use
17) US Pharmacopeia (USP) 35, Method 1111 Microbiological examination of non-sterile products: Acceptance Criteria for pharmaceutical preparations and substances for pharmaceutical use
21) ISO 29621, Cosmetics – Microbiology – Guidelines for the risk assessment and identification of microbiologically low-risk products, June 2010

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