

A guidance document on microbiological control of cosmetic products

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Preface

The purpose of this guideline is to help those concerned with the production or import of cosmetic products to maintain a good microbiological quality all through the life of the product. The guideline was prepared for the Danish Environmental Protection Agency (DEPA) in 2007-2009 by DHI Water, Environment and Health (DHI).

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Sammenfatning og konklusioner

En guide til mikrobiologisk kontrol af kosmetikprodukter blev udarbejdet via et projekt, som blev finansieret af Miljøstyrelsens Virksomhedsordning. Formålet med guiden er at introducere passende testmetoder og at øge kendskabet til de generelle krav inden for mikrobiologiske testlaboratorier. Guiden anbefaler, at man anvender de nyligt offentliggjorte ISO standarder, der er specielt beregnet til mikrobiologisk kontrol af kosmetiske produkter, før, i løbet af, og til slut i processen. Da der endnu ikke foreligger en ISO standard for provokations testning, præsenteres en provokationstest baseret på tidligere beskrevne metoder. Den beskrevne provokationstest er ikke blevet efterprøvet, og derfor har man ikke kendskab til, hvordan den fungerer i de forskellige laboratorier. Til trods herfor fandt vi det vigtigt at medtage en provokationstest, og den skal ses som et forslag til, hvordan en sådan test kan designes..

Summary and conclusions

A guidance document on microbiological control of cosmetic products was created within a project from Virksomhedsordningen of the DEPA. The intention of the guidance document is to introduce adequate methods of testing and knowledge of the general demands on microbiological testing laboratories. The guidance document recommends the use of the newly published ISO standards especially produced for microbiological control of cosmetic products, before, during and at end of use. As no ISO standard for challenge testing is yet available a challenge test based on earlier described methods is presented in this guidance document as a suggestion to how a challenge test can be constructed. Unfortunately the proposed challenge test has not been validated or tested in laboratories and therefore we do not have the knowledge of how it performs in different laboratories.

1 Introduction

The purpose of this guideline is to help those concerned with the production or import of cosmetic products to maintain a good microbiological quality all through the life of the product. Cosmetics refer to products intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition. For definition of cosmetic product in Danish see Kosmetikbekendtgørelsen, Kap.1 §3 (1) and in English see Article 1 in Council Directive 76/768/EEC (2). Contamination of cosmetics – during the production process – can cause adverse effects when used by sensitive individuals. A cosmetic product placed on the market must not cause damage to human health. This guideline will describe and recommend validated methods for measuring microbiological contamination of the cosmetic product before, during and at end of use. This guideline will also recommend how laboratories used for self-control of cosmetics products can be equipped.

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1.1 Background

Due to the wide range of formulations, manufacturing procedures and conditions of consumer use, the control of microbiological growth in cosmetics is complex. Legislation in relation to microbiological growth in cosmetics is not detailed, and concerned bodies are working on developing more detailed standards. One stakeholder is PEMSAC (Platform of European Market Surveillance Authorities in Cosmetics). PEMSAC is cooperation between European authorities within cosmetics. PEMSAC has assigned a standardisation mandate to the European Standards Organisations (CEN) concerning Good Manufacturing Practices for cosmetics products, see appendix 1. GMP is supposed to ensure that products, that are not necessarily sterile contain no harmful organisms and that the benign population is of low and stable order and/or declines over the product lifetime. At the same time International Organization for Standardization, technical committee for cosmetics, ISO/TC 217 is working on a series of standards for the detection and identification of microorganisms in cosmetic products. Denmark has no representative in this technical committee. The existence of these new standards will help create safe cosmetic products.

1.2 Legislation

A cosmetic product is regulated in Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (Cosmetics Directive). More than 55 amendments and adaptations have changed the Cosmetic Directive through the years. The Cosmetics Directive introduces a legal responsibility for companies assuring that products reaching the market place are not only microbiologically safe but will also continue to be safe throughout the products life. In close co-operation with Member States the Commission has issued a number of 'guidelines' to provide a coherent interpretation of various provisions of the cosmetics-Directive in the interest of Member States authorities and stakeholders, such as industry. The Cosmetics Directive is transposed into the individual national legal frameworks (law) and in Denmark it is implemented in the Statutory Order of the ministry of the Environment no. 422 of 4. May 2006 (Bekendtgørelse nr. 422 af 4. maj 2006 om kosmetiske produkter (1)). The Cosmetics and Medical Devices unit of the European Commission/Directorate General Enterprise and Industry is in charge of administering the Cosmetics Directive and supervises a correct implementation.

The cosmetic Directive consists of a body text and eight annexes:

- Indicative list of cosmetic product types
- Officially recognized symbols (two annexes)

The "negative lists"

- Substances prohibited in cosmetics (over 1200)
- Ingredients with limitation when used (over 150)

The "positive lists"

- Colorants with limitations
- Preservatives with limitations
- UV-filters with limitations

1.3 Definitions

A cosmetic product is defined as any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition.

1.4 Human Safety

The safety of a cosmetic product in the EU is the full responsibility of the manufacturer, the first importer into the EU market or the marketer. A cosmetic product put on the market must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use, according to Article 2 in the Cosmetics Directive and implemented in §10 in the Danish cosmetics regulation.

1.5 Dossier

The manufacturer or his agent or the person to whom a cosmetic product is manufactured or the person responsible for placing an imported cosmetic product on the Community market shall for control purposes keep a dossier readily accessible for inspection by the competent authorities of the Member State indicated by the address specified on the label. The dossier is not directly available in each Member State but only through the competent authority in the Member State, which the manufacturer or his agent specified on the label. The information required to produce a dossier is described in § 33 in the Danish cosmetics regulation (1) and in article 7a in the Cosmetics Directive 76/768/EEC (2). Each dossier must contain a safety assessment of the product and information on microbiological specifications of the raw materials used for production and in the product. Records should be maintained for all aspects of microbiological testing during development and manufacture of the cosmetic product. A guideline on Safety assessment of cosmetic products and how to comply a dossier is available in Guideline on safety assessment of cosmetic products:

<http://www.mst.dk/Udgivelser/Publications/2001/03/87-7944-336-2.htm> from the Danish EPA and in a Danish Version in Environmental Guideline No 9 2000. <http://www.mst.dk/Udgivelser/Publikationer/2001/03/87-7944-335-4.htm> Both guidelines can be found via the homepage of the Danish EPA.

1.6 Good Manufacturing Practice

Producers of cosmetic products are legally obliged to comply with the principles and guidelines of GMP. The requirements were formulated in Directive 93/35/EEC, the 6th amendment to the Cosmetics Directive. The ISO standard DS/EN ISO 22716:2007, Cosmetics - Good Manufacturing Practices (GMP) - Guidelines on Good Manufacturing Practices, gives guidelines for the production, control, storage and shipment of cosmetic products. These guidelines cover the quality aspects of the product, but as a whole they do not cover safety aspects for the personnel engaged in the plant, nor do they cover aspects of protection of the environment. The guidelines in

ISO 22716:2007 are not applicable to research and development activities and distribution of finished products. The standard can be acquired via Dansk Standards homepage, DS/EN ISO 22716:2007. COLIPA (3) and the Council of Europe (4) have also produced GMP guidelines.

GMP should ensure that products, whilst not necessarily sterile, contain no harmful organisms and that the microbiological population is of a low and stable order and/or declines over the product lifetime. GMP includes specific cleaning procedures to keep all apparatus and materials appropriately clean. Procedures also include microbiological control of raw materials, bulk and finished products, packaging material, personnel, equipment and preparation and storage rooms.

1.7 Durability Labeling

From March 2005 it was legally demanded (Directive 2003/15/EC, amending Directive 76/768/EC) to label durability on cosmetic products. This Directive is implemented as § 21 in the Danish cosmetics regulation.

Indication of the exact date of durability is not mandatory for cosmetic products with a minimum durability of more than 30 months. Instead, such products must be labelled with a symbol indicating the period of time in month/year after opening (PaO), for which the product can be used without any harm to the consumer. Such a symbol is the open jar found in annex eight of the Danish cosmetics Statutory:



Products with durability less than 30 months must be labelled with durability period and a fixed date: best used before the end of "insert date".

The manufacturer must have information supporting the microbiological stability of the product. The manufacturer must demonstrate that no unacceptable alterations of the product occur within the indicated durability period. Each product's PaO must be assessed with relevant methods. No official methodology is available but examples of sources of information for assessing a product's PaO may include:

- microbiological challenge tests
- stability data
- analytical data (e.g. preservative analysis)
- type of packaging
- experience with similar formulations and products
- consumer habits and practices.

2 Microbiological control

The different needs for microbiological examinations of cosmetic products are established from the microbiological risk analysis, which are carried out in order to determine the type of cosmetic product (low microbiological risk etc) you have. The microbiological risk analyses include consideration of the type of user, site of application, potential alteration of cosmetic products as well as the pathogenicity of microorganisms.

Specified microorganisms are aerobic mesophilic bacteria or yeast undesirable in a cosmetic product and recognised as a skin pathogen species that may be harmful for human health or as an indication of hygienic failure in the manufacturing process. Microorganisms considered as specified microorganisms are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Escherichia coli*.

2.1 Microbiological control of final product

No limits for microbial contamination of cosmetics are enclosed in the Cosmetics Directive or in the national Danish implementation of this Directive. Recommendations on limits of microbial contamination in cosmetic products can be found in the notes of guidance for the testing of cosmetic ingredients and their safety evaluation prepared by EU's Scientific Committee of Consumer Products (SCCP) (5). The Danish Environmental Protection Agency recommends the use of these levels.

In SCCP's notes of guidance, two separate categories of cosmetics products are defined, as various skin areas can be differently sensitive.

Category 1	Products specifically intended for children under 3 years, eye areas and mucous membranes, leave-on products.
Category 2	Other products, rinse-off products.

The quantitative specifications are – Generally acceptable levels:

Category 1	Total viable count for aerobic mesophilic microorganisms (bacteria, yeast and moulds) not more than 10^2 cfu/g or ml in 0,5g or 0.5 ml of the product
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Category 2	Total viable count for aerobic mesophilic microorganisms not more than 10 ³ cfu/g or ml in 0,1g or 0.1 ml of the product
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It is not acceptable that the following potentially pathogenic microorganisms are present in cosmetic products:

- ◆ *Staphylococcus aureus*
- ◆ *Pseudomonas aeruginosa*
- ◆ *Candida albicans*

The occurrence of indicator bacteria is not mentioned in SCCP's notes of guidance (5). But it is generally acknowledged that neither the occurrence of *E. coli* nor other members of *Enterobacteriaceae* are acceptable in cosmetic products.

Qualitative limits:

Category 1	<i>Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans</i> or <i>E.coli</i> must not be detectable in 0.5g or 0.5 ml of the product.
Category 2	<i>Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans</i> or <i>E.coli</i> must not be detectable in 0.1g or 0.1ml of the product

2.2 Microbial contamination

Microbiological durability depends on product composition, content of preservatives, manufacturing hygiene, packaging, transport and storage. The ability of microorganisms to grow and reproduce in cosmetic products is well known. Water is essential for microbial growth and water-based products often have a limited durability, as they are sensitive to microbial growth. More are cosmetics ideal nutrient media for microorganisms.

2.2.1 During manufacturing

Contamination during production and filling in cosmetic products may occur.

Raw materials can contribute to a significant level of microbial contamination to the finished product. Testing of raw materials before use, especially those of natural origin is important. The specifications of the raw materials must include microbiological purity. Water is a raw material, and the most common ingredient. Water must be tested continuously for microbial growth. It might be necessary to sterilise deionised water to obtain a sufficient purity.

Many other conditions of production may influence the contamination during manufacturing, such as contaminated areas, insufficient manufacturing hygiene, personnel hygiene and insufficient preservation. Effective cleaning is very important.

2.2.2 After opening

From the moment of opening the cosmetic product is subject to constant and variable microbial contamination from the domestic environment and the consumer's hands and body (the skin). Since microorganisms are ever present in the home, especially in warm, moist areas, such as bathrooms and kitchens, cosmetics are exposed to contamination with both spoilage and potentially hazardous micro-organisms during use.

Purity after opening depends on the preservative ability of the product, suitability of the packaging, storage and application.

The following scenarios can contribute to contamination of a cosmetic product, fingers dipped in product, spillage of water into product, shampoo used by several different people

2.3 Preservation

The function of preservation is for consumer protection and prevention of spoilage during normal and reasonable product use. The preservatives inhibit the growth of contaminating microorganisms during manufacturing, storage and use by consumers after opening.

The preservative efficacy of a formulation cannot be predicted in every detail and must be confirmed by microbial challenge testing (see section 4.4) since the activity of the preservative is dependent on the effect of individual ingredients and the packaging in which it is stored. Preservatives must be used at the lowest concentration that ensures their efficacy and this must be determined during the product development process.

The efficacy of antimicrobial preservation in cosmetics can be tested with the Challenge test.

The use of preservatives in cosmetics cannot replace good manufacturing practice.

3 Laboratories

In Denmark, there is no specific regulation on quality requirements or accreditation of microbiological laboratories used in self-control of cosmetic products. This gives the producer of cosmetic products freedom but also responsibility. When conducting microbiological examinations of cosmetic products it is necessary to pay attention to personal hygiene and to use appropriate working techniques to ensure that only those microorganisms that are present in the samples are enumerated. To help producers with their own laboratories for self-control of cosmetics products establish a reasonable laboratory quality level, the following chapter on quality management is included. Matters related to the working environment are not covered. Please refer to the Danish Working Environment Authority Guideline C.0.18.

3.1 Quality Management

3.1.1 Quality management standards

Quality management is defined in ISO 9000 (6) as coordinated activities to direct and control an organisation with regard to quality. Quality management generally includes establishment of a quality policy with quality objectives, and quality planning, quality control, quality assurance and quality improvement. For laboratories, ISO 17025 (7) specifies the general requirements for the competences of testing and calibration laboratories, and its implementation will ensure compliance with ISO 9000 as well. ISO 17025 describes both management requirements and technical requirements.

If your company has implemented a quality management system in accordance with ISO 9000, all management requirements and some of the technical requirements in ISO 17025 are already implemented and will be easy to extend to the laboratory. In this case it is recommended to fully implement ISO 17025.

If your company has implemented a Good Manufacturing Practice according to DS/EN ISO 22716, a number of both management and technical requirements similar to those of ISO 17025 are implemented and will be easy to extend to the laboratory. In this case it is recommended to follow the ISO 17025 as close as reasonable for the specific laboratory.

Below, we describe the recommended minimum requirements for the control laboratory.

3.1.2 Approach to quality management in microbiology laboratories

A common approach for implementation of a practical QA/QC system is “the 5 D’s”.

- **Decide** where it is relevant to perform quality management
- **Describe** who does what, how and when
- **Do** what is decided and described
- **Document** what has actually been done
- **Deem** whether procedures and practices give the desired results and make improvement, if necessary

In the following paragraphs each of the D’s are described and suggestions are given on how to do the D’s.

3.1.2.1 Decide

In order to decide where it is relevant to perform quality management it is suggested first to draw a flowchart of the analytical flow from sampling to reporting the results. An example of a flowchart is seen in Figure 1.

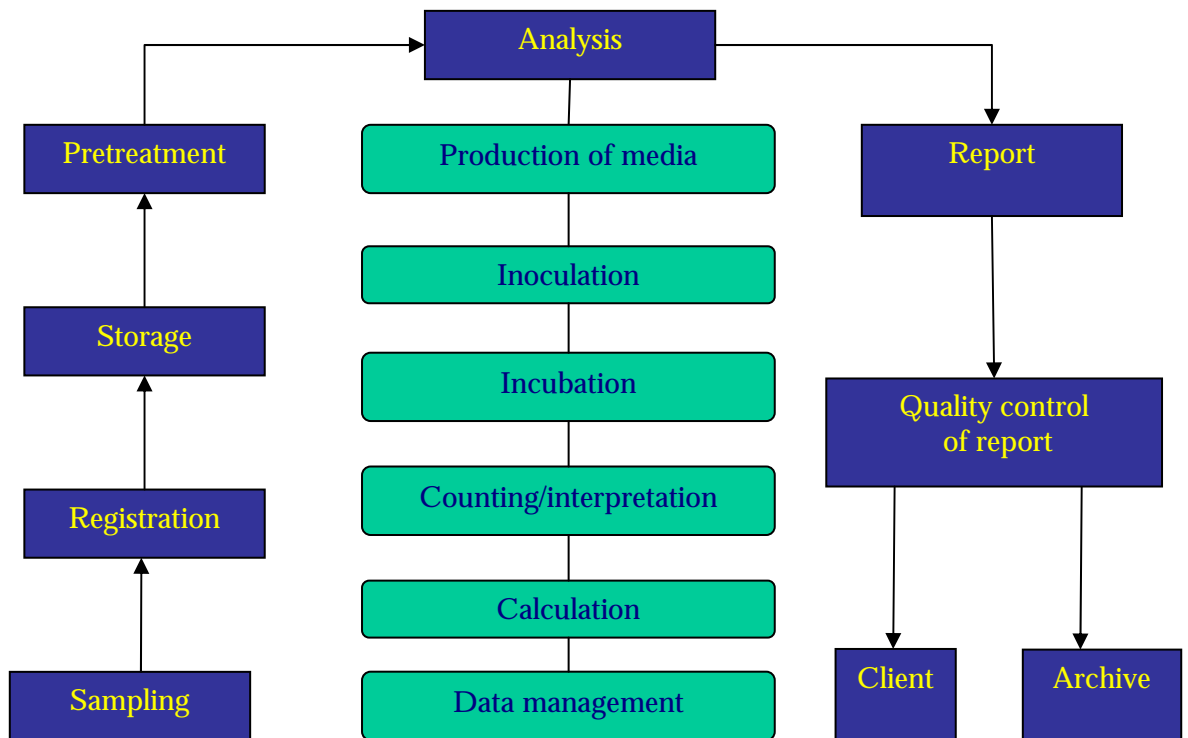


Figure 1: Example of a flowchart describing the steps of a microbial analysis.

The flow chart should identify all the critical steps of an analysis. The idea is now to carefully go through each step and decide which of steps needs a written operational procedure. In some cases more than one step may be needed. It is important to do this very carefully because missing procedures may hamper the analytical quality. On the other hand, the number of procedures should be limited to a minimum; because the more procedures to be followed the higher the chance is that one or more of them are not followed.

Deciding where to perform quality management and in what detail is a delicate balance. For instance the temperature is a critical factor in a microbial analysis, and a number of questions arise on how to manage the temperature. For instance: should the incubator temperature be monitored weekly, daily or recorded continuously? Is it OK to monitor the incubator temperature in one point, or should the spatial variation also be known? Should the thermometer be calibrated at each °C at all the temperatures, where the thermometer is used, or is it OK to intra- or extrapolate from two calibration points? The art of implementing good quality management is to find a satisfactory level of QA/QC without overdoing it. As a rule of thumb, procedures or devices that directly influence the analytical results should be given the highest priority.

3.1.2.2 Describe

When the steps requiring an operational procedure have been decided, the procedures should be written as short and clear as possible without missing any points. The procedures may be very simple consisting of a reference to a standard, for instance the analytical methods or sampling procedures, a reference to a maintenance manual, or it may be procedures entirely written by the laboratory, for instance procedures for control of incubators or thermometers. The procedures may have annexes such as templates for sample registration. A number of the procedures may be shared with other laboratories in the company, such as procedures for registration of chemicals, and calibration of pipettes and balances. It is suggested that the procedures follow a common format, which includes at least the following:

1. A unique title
2. The purpose of the procedure
3. The process
4. Responsibilities
5. Name of the author and the approving person
6. Date of approval, date of expiry and edition.

3.1.2.3 Do

This part is quite simple: You just have to do what was decided and described. However, this is also the hard part. The experience shows that procedures are often forgotten and left alone in the binder or in the drawer. A few things can be done to reduce the likelihood of forgotten and unfollowed procedures:

- The procedures should be reviewed by the staff doing the work to assure that the procedures are practical and in accordance with the way the work is actually carried out in the lab (assuming the work is done in a proper way).
- The procedures should be readily available to all relevant staff.
- A year plan for maintenance and calibration should be made and followed, and made readily available (e.g. posted on the wall).
- Education of and discussion among the staff members.

3.1.2.4 Document

Document what has actually been done. This requirement is included for at least four reasons:

- It provides a tool for identifying errors and thereby preventing the same errors to take place in the future work.
- It enables your company to perform internal audit to verify that the actions to be taken were actually taken.
- It enables audit to be done by an independent third party, if necessary.
- In case of complaints, or if unusual results have been obtained, the laboratory can control and prove that the quality of the analysis is sufficient and the results are reliable.

The requirement for documentation covers all operations that may influence the quality of the analysis. In a good QA/QC system, all relevant (and only the relevant) operations are described in the procedures, and must be documented. The best way is to provide templates where the work carried out can be recorded. Examples of documentation are signed templates for sampling, control and calibration of volumetric equipment, substrate control, employees' education, quality control of reports etc.

A short rule of thumb is: it must be possible for an auditor by a signed document to verify that a certain operation has been performed when and by whom.

3.1.2.5 Deem

Even the best QA/QC system can be improved and fine-tuned. Therefore it is necessary to evaluate the system periodically. This is done through several methods.

One of the methods is audit, internal (and external), for which requirements usually are laid down in the quality management /GMP system. During the audits, inconsistencies between procedures and the actual work are identified. It must be decided in each case, if the procedure or the practise should be changed.

Another method is internal quality control, which is a program carried out by the laboratory to show that the variability is under control, using tools such as standards, replicate samples and participation in proficiency tests. If the variability is deemed to be too high, actions must be taken to improve the procedures.

A third mechanism, and maybe the most important, is the daily discussions among the staff and with colleagues from other laboratories. It is important to encourage open discussions between all lab employees, and to be willing to make changes accordingly.

3.1.3 Requirements to quality management

This section describes a recommended minimum of requirements to the microbial control laboratory. The recommendations are based on ISO 17025, “EA - 4/10 - Accreditation in Microbiological Laboratories” (8) and DS/EN ISO 22716. However, full implementation of ISO 17025 is preferable to obtain consistently reliable results.

3.1.3.1 Document control

The laboratory must establish and maintain procedures to control all documents related to the quality of the analyses. These procedures should follow the requirements of ISO 17025 or ISO 22716.

3.1.3.2 Personnel

The laboratory management must ensure the competences of all personnel involved in planning, performing, interpreting and reporting of tests and/or calibrations. Testing and calibration must be performed or supervised by an experienced person with a degree in microbiology or equivalent, or with extensive relevant experience. The staff must have relevant practical working experience and have received adequate training in basic techniques such as plate pouring, counting of colonies, aseptic techniques etc.

Where a method or a technique is not regular in use, verification of personnel performance before testing is undertaken may be necessary. Critical interval between performances of tests should be established.

The laboratory must maintain job descriptions and documentation of staff qualifications.

3.1.3.3 Environment

The laboratory must ensure that the environmental conditions do not invalidate the results or adversely affect the required quality of any measurement.

The laboratory must monitor, control and record the environmental conditions as required by the relevant methods or procedures or when they influence the quality of the result. Due attention must be paid, for example, to biological sterility and temperature.

The laboratory should be arranged so as to minimise the risks of cross contamination. This can be achieved for example by constructing the laboratory according to the “no way back” principle, where all samples and cultures only travel in one direction through the laboratory. For instance, cultures or incubated plates should never enter media and sample preparation rooms. Alternatively, activities can be separated by time and space and appropriate precautions can be taken to ensure test and sample integrity, such as use of sealed containers and hygienic practises.

It is good practice to have separate locations or clearly designated areas for:

- sample receipt and storage
- sample preparation and challenge test preparations including sterile room or sterile cupboards. Powdery products should be handled separately.
- incubation and sample examination

- media and equipment preparation including sterilisation
- decontamination

The area for washing after decontamination may be shared with other laboratories provided that transfer of substances that could adversely affect microbial growth is prevented.

Space should be sufficient to allow work areas to be kept clean and tidy.

Rooms should be appropriately ventilated and at a suitable temperature.

Reduction of contamination may be achieved by having:

- smooth surfaces on walls, ceilings, floors and benches. Tiles are not recommended as bench covering material
- concave joints between the floor, walls and ceiling;
- minimal opening of windows and doors while tests are being carried out
- sun shades placed on the outside;
- fluid conveying pipes not passing above work surfaces unless placed in hermetically sealed casings
- a dust-filtered air inlet for the ventilation system;
- separate hand-washing arrangements, preferably non-manually controlled;
- cupboards up to the ceiling;
- no rough and bare wood;
- wooden surfaces of fixtures and fittings adequately sealed;
- stored items and equipment arranged to facilitate easy cleaning;
- no furniture, documents or other items other than those strictly necessary for testing activities.

There must be a cleaning programme for laboratory fixtures, equipment and surfaces.

Laboratory coats must be worn in the laboratory and must be removed before leaving the area.

Access to the microbiology laboratory should be restricted to authorised personnel.

See also ISO 21148 General instructions for microbial examination.

3.1.3.4 Test and calibration methods and method validation

The laboratory must use appropriate methods and procedures for all tests and/or calibrations. These include sampling, handling, transport, storage and preparation of items to be tested or calibrated, and where appropriate, an estimation of the measurement uncertainty as well as statistical techniques for analysis of test and/or calibration data.

If laboratory-developed methods or non-standard methods are used, they must be validated in house. Standard methods used on matrices not specified in the standard must be validated as well.

Validation of a microbial method requires a substantial amount of work. The standards for validation (DS/EN ISO 16140 or DS/ENV ISO/TR 13843)

should be followed. See also EA - 4/10. To avoid the validation procedure it is strongly recommended to use the ISO methods described in section 4. However, even when a complete validated method is used, the laboratory still needs to verify on a regular basis that performance can be met, e.g. by use of spiked samples or reference materials.

The laboratory must have and must apply procedures for estimating the uncertainty of measurements. Rigorous, metrologically and statistically valid calculation of uncertainty of microbial analyses cannot be performed. It is appropriate to base the estimate of uncertainty on repeatability and reproducibility data, combined with bias estimation from participation in proficiency testing or use of standard materials when possible. The individual components of uncertainty must be demonstrated to be under control and their contribution to the variability of the evaluated results. Some components such as pipetting, weighing and dilution effects can be readily measured and evaluated. Other components such as sample stability and sample preparation cannot be evaluated in a statistical manner but their importance should be considered. See also section 3.1.3.6

Calculations and data transfers must be subject to appropriate checks in a systematic manner.

3.1.3.5 Equipment

The laboratory must be furnished with all items of sampling, measurements and test equipment required for the correct performance of the tests.

The laboratory must operate a documented programme for the maintenance, calibration, and performance verification of its equipment.

Maintenance of essential equipment must be carried out at specified intervals and detailed records must be kept.

The laboratory must establish a programme for the calibration and performance verification of equipment which directly influences the test result. Examples of calibration and performance checks are given in EA-4/10. Before taken into service, equipment must be checked to establish that it meets the requirements.

The temperature is an important parameter and the laboratory must have temperature measuring devices of an appropriate quality. Calibration of the devices must be traceable to national or international standards for temperature.

The stability of temperature and the uniformity of the temperature distribution in incubators, water baths and ovens must be established initially and documented with respect to typical uses. The initial validation must be checked and recorded after each significant repair or modification, and operating temperatures must be monitored and recorded.

Autoclaves must be capable of meeting the time and temperatures specified in the methods used. Pressure cookers only equipped with a pressure gauge are not acceptable. Initial validation must include operating cycles and load configurations used in practice. Temperature sensors should be positioned inside containers filled with liquid.

Monitoring of autoclaving should be carried out using a thermocouple and a recorder to produce a chart or printout, or by the use of chemical or biological indicators. Autoclave tape and indicator strips should only be used to show that the load has been processed.

Balances and weights must be calibrated traceably at regular intervals.

Laboratories must carry out initial verification of volumetric equipment and make regular checks to ensure that the equipment is performing with the required specification. Verification should not be necessary for glassware which has been certified to a specific tolerance. Equipment should be checked for the accuracy of the delivered volume against the set volume (for several different settings in the case of variable volume instruments) and the precision of the repeat deliveries should be measured. Single use disposable volumetric equipment should be obtained from ISO 9000 certified manufacturers. An initial validation of the suitability of the equipment must be carried out.

Conductivity meters, oxygen meters, pH meters and other similar instruments should be verified regularly or before each use. The buffers used for verifications purposes should be stored in appropriate conditions and should be marked with an expiry date, and their use documented.

3.1.3.6 Reagents and culture media

Laboratories must have procedures for selecting and purchasing of the services and supplies it uses that affect the quality of the tests. To ensure that the quality of the reagents and media used is appropriate for the test concerned, it is recommended to obtain reagents and media from ISO 9001 certified manufacturers. In this case an initial validation of the suitability of the equipment must be carried out. In case in-house prepared media or ready-to-use media from non-certified manufacturers are used, each batch should be validated according to ISO 11133.

Laboratories must ensure that all reagents (including stock solutions), media, diluents, and other suspending fluids are adequately labelled to indicate, as appropriate, identity, concentration, storage conditions, preparation date, validated expiry date and /or recommended storage periods. The person responsible for preparation should be identifiable from records.

The laboratory must keep a record of all purchased reagents, media etc.

3.1.3.7 Internal quality control

Internal quality control consists of all the procedures undertaken by a laboratory for the continuous evaluation of its work. The main objective is to ensure the consistency of results day-to-day and their conformity with defined criteria.

A programme of periodic checks is necessary to demonstrate that variability (i.e. between analysts and between equipment and materials etc.) is under control. All tests included in the control of the products need to be covered. The programme may involve:

- the use of spiked samples
- the use of reference materials (including proficiency testing scheme materials)
- replicate testing

It is recommended to follow option 1 described in ISO/TS 19036:2006 (9).

A laboratory may use a test at rare. It is recognised that in such cases an ongoing internal quality control programme may be inappropriate and that a scheme for demonstrating satisfactory performance which is carried out in parallel with the testing, may be more suitable.

3.1.3.8 External quality assessment (proficiency testing)

If available laboratories should regularly participate in proficiency testing which are relevant to their activities, bias should be assessed and the validity of the whole quality system should be checked.

3.1.3.9 Internal audit

The company must periodically, and in accordance with a predetermined schedule and procedure conduct internal audits of the activities of the control laboratory to verify that its operations continue to comply with the requirements of the laboratory quality management system. The internal audit programme must address all elements of the laboratory management system. Internal audit is a requirement of both ISO 9001 and ISO 22716 and can be extended to cover the laboratory as well. The audit must be carried out by specially designated personnel having both quality management competence and technical competence. If the company does not have independent technical competence, external technical advisors can be included in the audit.

Internal audit follow-up must confirm the satisfactory completion of the audit or satisfactory implementation of corrective actions.

The area of activity audited, the audit findings and corrective actions that arise from them must be recorded.

4 Analytical Methods

Through its technical committee for Cosmetics ISO/TC 217, the international organization for standardization (ISO) has presented a number of new international standards for microbiological examination of cosmetic products. These standards are detailed and cover the needs of a large part of available cosmetic products. It is strongly recommended to incorporate the use of ISO standards in microbiological testing of cosmetic products. In the following some of these standards are presented.

In general, the efficacy of antimicrobial preservation in cosmetics can be tested by the Challenge test. The test shows the ability of the cosmetic product to reduce the count of micro-organisms after a contamination. Challenge testing is mandatory for all cosmetic products that under normal conditions of storage and use may deteriorate or form a risk to the consumer. As neither a legal nor a universal challenge test method is available; it is up to the manufacturer to decide on the details of the test to be used.

4.1 Standards under development

The technical committee for Cosmetics ISO/TC 217 is developing two new guidelines. The laboratories should be updated on the status of the standards.

ISO/CD 29621 is a guideline for the risk assessment and identification of microbiologically low-risk products. In the committee draft stage the guideline has been approved for registration as a draft international standard.

ISO/NP 11930 is general information on evaluation of the antimicrobial protection and has been approved as a new project.

4.2 Neutralization and preparation of water-immiscible samples.

Microbial examination of cosmetics has at least two inherent problems, namely the toxic properties of the conservation systems and water-immiscibility of some products. These problems are very important to deal with in order to achieve a correct result.

Cosmetic products are usually conserved to maintain a hygienically good quality during storage and to prevent growth of microorganisms during use of the product. The conservation system is likely to inhibit growth on agar plates or in enrichment broths and thereby likely to give rise to false negative results. Therefore the conservation system must be inactivated or neutralized before analysis.

The initial steps of microbial examinations involve preparation of an initial suspension of the microorganisms in the sample. This suspension is subsequently diluted to achieve sub-samples with appropriate concentrations of microorganisms. If the cosmetic product to be tested is water-immiscible,

the diluents should contain a suitable amount solubilising agents such as Polysorbat 80.

Samples with antimicrobial properties must be neutralized before analysis. This is done by adding neutralizers to the diluents. Relevant neutralizers are suggested in the specific standards (see section 4.3) and in ASTM E 1054 (10). In all cases and whatever methodology, the neutralization of the antimicrobial properties of the product must be checked and validated. The validation procedures are described in each specific standard. The principle of the validation procedure is to add a known amount of the relevant test strain(s) to the initial sample suspension and compare the number of micro-organisms with a control without the sample. In case of qualitative or presence/absence tests, growth and characteristics of the colonies are examined.

4.3 Examination of microbial quality of products

Cosmetic products must be subjected to microbiological control as described in Chapter 2. A number of ISO standards have been developed to give guidelines for the manufacturers. It is recommended that all laboratories use the ISO standards described in this section.

4.3.1 ISO 21149 Cosmetics – Microbiology – Enumeration and detection of aerobic mesophilic bacteria

The standard contains guidelines for enumeration and detection of mesophilic aerobic bacteria in cosmetics by counting colonies on agar medium after aerobic incubation or by checking absence of bacterial growth after enrichment.

4.3.2 ISO 18415 Cosmetics – Microbiology – Detection of specified and non-specified micro-organisms.

The standard contains guidelines for the detection and identification of specified microorganisms in cosmetic products as well as for the detection and identification of other kinds of aerobic mesophilic non-specified microorganisms in cosmetic products. The standard contains guidelines for the detection of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, or *Candida albicans*. The detection is carried out by enrichment in a non-selective broth followed by isolation and identification. The identification consists of gram staining, catalase- and oxidase test followed by the use of an identification test kit.

4.3.3 ISO- methods for the detection of specific microorganisms: *E. coli* (ISO 21150), *Pseudomonas aeruginosa* (ISO 22717), *Staphylococcus aureus* (ISO22718) and *Candida albicans* (ISO 18416).

In all four standards, the first step is enrichment in a non-selective broth to increase the number of microorganisms without the risk of inhibition by the selective ingredients present in the growth media. The second step is isolation on selective media followed by identification tests.

4.3.4 ISO/FDIS 16212 Cosmetics – Microbiology – Enumeration of yeast and mould.

This standard is currently available as a draft. The method involves enumeration of colonies on Sabouraud dextrose chloramphenicol agar medium. Enumeration may be carried out as a pour plate, surface spread or membrane filtration method.

4.4 Efficacy of preservation – Proposal for a Challenge test-not validated

A challenge test is a procedure in which a product is challenged by exposure to specified types of bacteria and fungi. The product is then incubated at a given temperature, samples are taken at specified intervals and the number of microorganisms is determined. Normally the product is challenged to the microorganisms *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, but in-house microorganisms, found as contaminations in the products, may be used for additional specific purposes of challenge testing. The antimicrobial properties of the product are acceptable, if a significant decrease or no increase in viable count of micro-organisms is seen, when the product is tested under consideration to storage and use.

The challenge test should be performed both during development of the preservative system and as an evaluation of the protection efficacy in intact, in-use or in ending cosmetic products.

There are no approved ISO standards available for challenge tests. Until the new ISO standard is available, the procedure below in section 4.4.1 is recommended. The procedure has not been validated why it is suggested to perform an in-house validation before use. Similar challenge tests are described in the European Pharmacopoeia (11), the US Pharmacopoeia (12) and in ASTM (the American Society for Testing and Materials) (13). The methods are similar but differ in the detailed procedures, test organisms, criteria for passing the test and requirements for validation. Inexperienced laboratories should send the samples to an accredited laboratory. Another alternative is to contact the producer/deliverer of the preservative; they can usually provide laboratory capacity.

4.4.1 Proposed procedure for challenge testing

The product is challenged with cell/spore suspensions (10^8 cells/spores pr. ml) at a concentration of 10^5 – 10^6 cells/spores pr. ml. The challenged product is incubated at $22\text{ °C} \pm 1\text{ °C}$ in the dark. Samples for determination of plate counts are taken after 0, 7, 14, 21 and 28 days.

Challenge the product with

- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Candida albicans*
- *Aspergillus niger*

Other relevant organisms, such as commonly observed contaminating organisms, should also be used as test organisms.

Use Lethen agar which contains the neutralisers polysorbat 80 and lecithin, or Casein soya bean digest agar for bacteria, and Saboraud-glucose agar without antibiotics for fungi.

The initial plate counts are determined immediately after addition of the test organisms. The concentration of bacteria should reach a log 3 reduction after 14 days, and there should be no increase in the concentration after day 14. The concentration of fungi should reach a log 2 reduction after 14 days and there should be no increase in concentration after day 14.

The efficacy of the neutralisers shall be validated according to the specific ISO standards (se section 4.3) or according to ASTM E 1054.

5 References

1. BEK nr 422 af 04/05/2006 (retsinfo.dk)
2. Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.
3. Colipa guidelines; cosmetic good manufacturing practices, 1994 (pdf can be downloaded free of charge from colipa.com).
4. Van der Maren L. Guidelines for good manufacturing practice of cosmetic products (GMPC). Council of Europe, Health protection of the consumer, ISBN: 98-871-2849-9 (1995).
5. The SCCP's notes of guidance for the testing of cosmetic ingredients and their safety evaluation 6th revision December 2006 link.
6. Standards ISO 9000:2005 or ISO 9001:2000 can be obtained from Dansk Standards net shop.
7. Standard ISO 17025:2005 can be obtained from Dansk Standards net shop.
8. EA-4/10 (rev.2) Accreditation for Laboratories Performing Microbiological Testing can be downloaded from <http://www.european-accreditation.org>
9. ISO/TS 19036:2006 Microbiology of food and animal feeding stuffs -- Guidelines for the estimation of measurement uncertainty for quantitative determinations
10. E 1054 – 02 Test Methods for Evaluation of Inactivators of Antimicrobial Agents astm.org. New version ASTM E1054 - 08 Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents
11. European Pharmacopoeia Commission Efficacy of antimicrobial preservation. Strasbourg, France: European Pharmacopoeia Commission; European Pharmacopoeia EP 5.1.3, 1997
12. U.S. Pharmacopeia. USP XXI
13. E 640 – 78(1998) Test Method for Preservatives in Water-Containing Cosmetics astm.org New version ASTM E640 - 06 Standard Test Method for Preservatives in Water-Containing Cosmetics.



EUROPEAN COMMISSION
ENTERPRISE AND INDUSTRY DIRECTORATE-GENERAL

Chemicals and construction
Chemicals

Brussels, 28th November 2005
M/375

STANDARDISATION MANDATE ASSIGNED TO CEN CONCERNING GOOD MANUFACTURING PRACTICE FOR COSMETICS PRODUCTS

1. MOTIVATION

This standardisation mandate relates to Council directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (hereinafter the Cosmetics Directive). The directive based on article 95 of the Treaty aims to insure free circulation of cosmetic products into the Community market. To that end it determines at Community level the regulations which must be observed as regards the composition, labelling and packaging of cosmetic products

According to article 7a (1) of the Cosmetics Directive *“the manufacturer or his agent or the person to whose order a cosmetic product is manufactured or the person responsible for placing an imported cosmetic product on the Community market shall for control purposes keep [inter alia] readily accessible to the competent authorities [...] the method of manufacture complying with the good manufacturing practice [...].*

However, no good manufacturing practice in the cosmetic sector is currently defined at Community level. In order to avoid unnecessary legislation and in view of better regulation and simplifying Community legislation, creation of a standard in this area would be the best approach. Indeed the standard would allow to relate to a common reference in this technical field without creating burdensome and avoidable legislation.

2. DESCRIPTION OF THE MANDATED WORK

The Commission invites the ESO to establish a European standard giving guidance for the production, control, storage and shipment of cosmetic products.

For the purpose of this mandate, “Cosmetic products” shall mean *“any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition. (article 1 of the Cosmetics Directive)”*.

In order to facilitate a wide acceptance of the standard, the ESO will take into account, as much as possible, the work undertaken by the international standards organisations on the same subject, and, in particular, the standard(s) or other standardisation deliverables under preparation or published as a result of ISO/TC 217 “Cosmetics”, particularly the draft under preparation under reference ISO/CD 22716 “Cosmetics - Good manufacturing practice

(GMP)". The ESO will avoid any unnecessary duplication of work with the international standards organisations, particularly by using the provisions for parallel approval procedures provided for in the existing co-operation agreements ("Vienna Agreement")

3. BODIES TO BE ASSOCIATED

As appropriate, the ESO will ensure that the representative organisations of consumers interests (ANEC), environmental protection (ECOS), workers (ETUI-REHS), small and medium-size enterprises (NORMAPME) and every relevant industrial organisation, in particular COLIPA¹, take part in the elaboration of the standard.

4. EXECUTION OF THE MANDATED WORK

The ESO will deliver a draft European standard and submit it to a public enquiry by 2006-02-28.

The ESO will publish a final European standard by 2007-08-31. By that date the standard will be available in English, French and German, and the correct title of the standard will be available in the other Community languages.

At the latest six months after the publication of the European standard by the ESO, it will be implemented as a national standard by all national standards institutes in all Member States and every conflicting national standard will be withdrawn.

The acceptance of this mandate by one of the ESO will trigger the standstill period referred to in Article 7 of Directive 98/3/EC of 22 June 1998.

¹ The European Cosmetic Toiletry and Perfumery Association.

Challenge test of water miscible cosmetic products

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1.1 Purpose

The purpose of this procedure is to determine the effectiveness of antimicrobial preservatives used to stop proliferation or to prevent microbial contamination in cosmetic products.

The procedure is particularly useful during development of new products.

1.2 Principle

The cosmetic product is challenged by adding 10⁵-10⁶ CFU/ml or g of a single strain of test microorganism via cell suspensions of approximately 10⁸ cells/spores pr. ml and is incubated at 20-25° C protected from light. After 0, 7, 14, 21 and 28 days of incubation, samples are taken to determine the number of microorganisms by plate count. The product will pass the test, if the analyses are valid and results are in compliance with the acceptance criteria.

1.3 Scope

This procedure can be used for water miscible products.

1.4 References

European Pharmacopoeia (6.0). Method 5.1.3 Efficacy of antimicrobial preservation. 01/2008:50103.

ASTM E 640 – 78 Standard test method for preservatives in water containing cosmetics. American Society for Testing and Materials, 1991 (reapproved 1998). Annual Book of ASTM Standards. ASTM, Philadelphia, Pa.

ISO 16212:2008 Cosmetics – Microbiology – Enumeration of yeast and mold. First edition 2008-10-07.

ISO 22149:2006 Cosmetics – Microbiology – Enumeration and detection of aerobic mesophilic bacteria. First edition 2006-03-01.

ISO 22717:2007 Cosmetics – Microbiology – Detection of *Candida albican*. First edition 2007-07-15.

ISO 22717:2006 Cosmetics – Microbiology – Detection of *Pseudomonas aeruginosa*. First edition 2006-02-01.

ISO 22718:2006 Cosmetics – Microbiology – Detection of *Staphylococcus aureus*. First edition 2006-02-01.

1.5 Equipment

Balance ($\pm 0.01\text{g}$)

Equipment for homogenisation (e.g. stomacher)

pH meter (± 0.1 pH units)

One autoclave to clean equipment and media, and another autoclave to treat contaminated waste

Incubators: 20-25 °C and 30-35 °C.

Other standard equipment for microbiological laboratories necessary to perform sterile work, sample handling and work with cultures.

1.6 Test organisms

Pseudomonas aeruginosa CCUG 22801 (ATCC 9027; NCIMB 8626; CIP 82.118)

Staphylococcus aureus CCUG 10778 (ATCC 6538; NCTC 10788; NCIMB 9518; CIP 4.83)

Candida albicans CCUG 19915 (ATCC 10231; NCPF 3179; IP 48.72)

Aspergillus niger CCUG 18919 (ATCC 16404; IMI 149007; IP 1431.83)

The organisms can be obtained from type culture collections.

1.7 Chemicals and substrates

For ***P. aeruginosa***, ***S. aureus*** and ***C. albicans*** a tryptone (1,0 g/l) sodium chloride (8,5 g/l) diluent is used.

For ***A. niger*** a tryptone (1,0 g/l) sodium chloride (8,5 g/l) solution with Polysorbat 80 (0.5 g/l) is used.

Agar for cultivation and quantification of ***P. aeruginosa***, ***S. aureus*** and ***C. albicans***. Lethen agar

Agar for cultivation and quantification of ***A. niger***: Saboroud-glucose (Saboroud-dextrose) agar without antimicrobials.

Lethen agar contains polysorbat 80 and lecithin, which inactivates many antimicrobial preservatives.

1.8 Procedure

1.8.1 Preparation of inocula

Prepare stock and working cultures of the test organisms as described by the supplier.

Before the test, inoculate the surface of the agar with recently grown stock or working cultures of each of the specified microorganisms. Incubate the agar plates with *P. aeruginosa* and *S. aureus* at 30 °C to 35 °C in 18 to 24 hours, agar plates with *C. albicans* at 20 °C to 25 °C in 48 hours, and agar plates with *A. niger* at 20 °C to 25 °C in 1 week or until good sporulation is obtained. Examine the agar plates for contamination before use.

To produce challenge suspensions, harvest the bacterial and fungal cultures by transferring colonies from the agar plates to diluents to a concentration of approximately 10^8 *P. aeruginos* or *S. aureus* pr. ml or approximately 10^7 *C. albicans* or *A. niger* pr. ml. Determine the number of colony forming units (CFU) pr. ml immediately after resuspension by plate count on the specified agar.

In order to make quick assessments of the concentration of organisms in the challenge suspensions, it is advised that the laboratory compares different concentrations of the specific test organisms (determined by plate count) in diluent against rapid detection methods, i.e. absorbance at 620 nm (typical absorbance of 10^8 bact/ml is 0.15 to 0.46 at 1 cm), turbidometry or visual comparison to McFarland standards.

1.8.2 Preparation of challenge test samples

Transfer 10 times 100 g or 10 times 100 ml of the product to be tested into sterile double Stomacher bags (one bag inside the other). Of these, two are used for each of the four test organisms and two are used as uninoculated controls.

Inoculate the test samples by adding no more than 1 ml of challenge suspension pr. 100 ml (or g) test product. Homogenise the test samples in the Stomacher for 30 seconds.

1.8.3 Sampling and incubation

Take a zero sample for each microorganism immediately after homogenisation (see 8.4) and incubate as described in 8.1. Incubate the Stomacher bags with the challenged test samples at 20 – 25 °C. It is important to seal the bags to avoid evaporation. Leave approximately 10 % headspace in the Stomacher bags.

Take further samples after 7, 14, 21 and 28 days. Homogenise before each sampling.

1.8.4 Analysis of samples

Transfer 10 g or 10 ml to 90 ml diluents (or 1 g/1ml to 9 ml). The density of the product must be known if the transfer is based on weight. Homogenise the dilutions and spread 100 µl of dilutions in duplicate on the surface of the agar. Use the 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} dilutions for the bacterial challenges and 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} for mold and yeast challenges at time zero. For subsequent sampling select the dilutions to be analysed on the basis of the results of the initial analyses. It is suggested to use the 10^{-1} , 10^{-2} and 10^{-3} on day 7 for *P. aeruginos* and *S. aureus* and 10^{-1} and 10^{-2} for *C. albicans* and *A. niger*. Incubate as described in **1.8.1**, count the number of colonies and calculate the plate count according to ISO 21149:2006, and plot the \log_{10} transformed results versus time.

1.9 Validity criteria

1.9.1 Validity of analyses

If the organisms grow readily on the plates, and if there is a 1:10 relation between the dilutions used for calculation of each analysis, the result of the analysis is valid. The relation between dilutions can be tested by a χ^2 test with 1 degree of freedom:

$$\chi^2 = \frac{(C1 - \frac{V1}{V2} C2)^2}{\frac{V1}{V2}(C1 + C2)}$$

where C1 and C2 are the number of colonies counted in dilutions V1 and V2. If $\chi^2 \geq 3.84$ then the dilution is significantly different from 1:10 at the 5% level. If a low dilution (more sample) has a relatively low count compared to a higher dilution (less sample) it may be caused by inhibition by the antimicrobial preservative in the tested product, and the test should be repeated with an alternative neutralizer. See for instance ISO 22717 annex B for information on alternative neutralizers.

1.9.2 Validity of test organisms and neutralising agent

If no growth is observed on the agar plates it may be caused either by poor viability of the test organisms or infectivity of the neutralising agent.

If no growth is observed, test organisms are spread in duplicate on the 10^{-1} and 10^{-2} plates and on clean control plates. Poor viability of the test organisms is shown, if no growth is observed on the control plates. In this case the entire test should be repeated with new and viable test organisms.

If no growth is observed on the 10^{-1} or 10^{-2} plates, and growth is observed on the control plates, then the neutralizing agent has been ineffective. The lab should try to wash away/neutralize the antimicrobial preservative with a sterile diluent to which an alternative neutralizer has been added. Again, the test organisms are spread on the plates. If no growth is observed on the washed plates and growth is observed on control plates treated in a similar way, then the antimicrobial preservative is accepted. If growth is observed, the test is repeated with an alternative neutralizer.

1.10 Acceptance criteria

The antimicrobial preservation system is accepted if:

the performed analyses were valid according to section 1.9.1, and

- bacteria are reduced by 3 \log_{10} units after 14 days, and no increase in plate counts after day 14.
- mold and yeast are reduced by 2 \log_{10} units after 14 days, and no increase in plate counts after day 14.

or if

no growth is observed after washing with alternative neutralizers according to section 1.9.2.