COSMETICS:
EFFICACY AND SAFETY TESTS

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A BRIEF OVERVIEW OF CHELAB

- Founded in Resana, Italy 1979
- Leader in Testing Services and Research
- Privately owned
- 600 employees
- 65000 Clients
OUR VISION

- We aim to be the most competitive and the most productive service organization in the central and southern Mediterranean.
- Our core competences in testing, inspection, verification and certification are being continuously improved to be best in-class.
- We chosen middle east near by Mediterranean market solely determined by our ability to be most competitive and deliver unequalled service to our customers.
CHELAB laboratories: FOOD, INDUSTRIAL & ENVIRONMENTAL, NON FOOD (cosmetics, detergents, packaging, textiles), PHARMACEUTICAL (GMP, GLP, REACH) and R&D.
ITALY LEADING INSPECTION, VERIFICATION, TESTING AND CERTIFICATION

- More than 600 scientists and employees
  - Europe: 600 employees
  - Middle East: 40 employees in Turkey
- A network of over 80 laboratories across central and eastern Europe
- Received the accreditation to conduct agriculture, environment, food-beverage, materials inspection and GMO analysis from the European Accreditation Body
- In 2011, the Turkish Ministry of Health recognized CHELAB as a laboratory authorized to inspect biocidal products for efficacy tests.
OUR IMPARTIAL SERVICES WORK ACROSS GOVERNMENTS AND INSTITUTIONS

- Improving visibility and accountability
- Improving import/export goods processing
- Reference lab in central Europe
- Support good governance
FINANCIAL HIGHLIGHTS & 2011 OUTLOOK

- Revenue growth of 15% to Euro 1.3 million
- 42.5 million Euro turnover in 2010
- Organic growth of 5%
- New laboratory enterprise system for better data management to drive efficiency and enhance quality
GEOGRAPHIES IN EUROPE
CHEMICAL ANALYSIS ON COSMETICS

1. Determination of preservatives
2. HPLC determination of EDTA
3. Lactic and citric ACID (organic acids)
4. ICP/MS determination of heavy metals
5. GC/MS determination of allergenic aromatizing substances
6. GC/MS determination of synthetic moss
7. HPLC determination of UV filters
8. Determination of N-nitrosodiethanolamine (NDELA)
CHEMICAL ANALYSIS ON COSMETICS

9. HPLC determination of panthenol
10. Biodegradability test on raw materials for cosmetics
11. HPLC and qualitative determination of dyestuffs
12. GC/MS quantitative determination of phthalates
13. Determination of amphoteric surfactants
14. Determination of glycerin and sorbitol
15. Determination of fluorinated compounds
16. Clorexidina and Benzalkonium chloride determination
17. Formaldehyde and releasable formaldehydes compounds
WHAT IS A COSMETIC PRODUCT
(Law 713/86 Art. 1)

COSMETIC PRODUCTS are those substances and preparations without medical properties destined to be applied on the surface of human body (skin, piliferous zone, nails, lips, external genitals) or on teeth and mouth mucous membrane to clean, perfume and modify their aspect.
COSMETICS
REQUIREMENTS

- DETERGING
- PROTECTION
- GOOD CONDITIONS of MAINTENANCE
- DEODORATION
- PERFUME
- DECORATION
- BEAUTY TREATMENTS
COSMETICAL ACTIVITIES (Law 713/86 Art . 2)

Cosmetic products do not have therapeutic properties.
AIM OF A COSMETIC

A COSMETIC product can help in the maintenance of skin health.

For this reason the action and the characteristics of a cosmetic should be limited to skin.
USE EFFICACY

A COSMETIC is a complex multi-factorial composition which includes different ingredients. The efficacy of a cosmetic cannot be guaranteed by a unique substance but from the combination of various ingredients.
USE SAFETY
(Law 713/86 Article 5)

Article 5 “Cosmetic products have to be produced, packed and sold in such a way to avoid the risk for human health when applied in normal conditions of use. For this reason they have to be considered the packaging, the labelling, the eventual operating instructions and the elimination of other information provided by the producer or by the selling superintendent of this product in the European market.”
PERFORMANCE TESTS

EFFICACY TESTS

They have the aim to verify the potential efficacy of the cosmetic preparations.

SAFETY TESTS

This test has the aim to guarantee the safety of use of the cosmetic product during its shelf life.
EEMCO GUIDELINES

"...The producer ... provides to the Ministry of Health…. for checking... the test results CARRIED OUT ON THE COSMETIC PRODUCT whether the nature of the product effect justifies it" Art. 10-ter Law 11.10.1986 n° 713

WHAT IS EEMCO

Guarantee of the method and measurements uniformity in the \textit{in vivo} tests for the cosmetic efficacy

Major reproducibility of data and truthfulness of the "marketing claim"

WHAT IS THE USE OF THE GUIDELINES?
EFFICACY TEST

On each kind of cosmetic product it is possible to perform specific *in vivo* and *in vitro* tests:

**SKIN CARE**
- HYDRATION
- TEWL
- MICRO-RELIEF ANALYSIS OF SKIN AND WRINKLES
- CELLULITE

**MAKE UP**
- MASCARA
- EYELINER
- LIPSTICK

**HEAR PRODUCTS**
- PERFORMANCE TESTS

**DEODORANTS**
- SNIFF TEST

SENSORIAL ANALYSIS AND CONSUMER TEST
HYDRATION TEST - short term

BETTER HYDRATION VALUE OF THE STRATUM CORNEUM

EFFICACY DECLARATION OF THE COSMETIC PRODUCT

Methods for the in vivo measurements of the aqueous content, on the basis of the ELECTRIC PROPERTIES of the STRATUM CORNEUM

INSTRUMENT: CORNEOMETER CM 825
The aim of the test is to evaluate the possible improvement of skin hydration after a hydrating cosmetic treatment.

CLAIM hydrates for X hours
Trans Epidermal Water Loss

STUDY ON THE BARRIER FUNCTION OF THE STRATUM CORNEUM THROUGH THE TRANS EPIDERMAL WATER LOSS

Instrument: Tewameter TM210

This instrument consists in a tube equipped with sensors for the detection of the relative humidity and temperature connected to a display showing the relative humidity values.

It is not an invasive technique, useful for the discrimination of the irritating effect of detergents

CLAIM NOT AGRESSIVE on skin
IN VIVO MICRO-RELIEF ANALYSIS OF SKIN AND WRINKLES

INSTRUMENT: DERMATOP BLUE

It is a last generation skin scanner able to visualize the skin structure comparing the results obtained by evaluating the volunteers at the time interval 0 and after a fixed period of product use.
DERMATOP BLUE

Z axis

X axis

-60°

60°
DERMATOP BLUE
DERMATOP BLUE

Skin in optically monitored by using a digitalization system of the image avoiding the use of silicone copy.

This system is based on the graphic representation of the in vivo skin, illuminated in a particular way. The obtained image is elaborated automatically and evaluated following clinical parameters, qualitatively and quantitatively corresponding to the physiologic condition of the skin surface.

This parameters derives from those used for wrinkledness.

In order to evaluate the skin before and after the treatment, the 2 more representative parameters (“2D-roughness statics”) are chosen for each volunteer and each time interval:

- \( R_z (\mu m) \) : Average Maximum profile height difference (Rptm)
- \( R_a (\mu m) \) : Linear average profile roughness
DERMATOP BLUE IMAGES

Before treatment (T0)

After 15 days of treatment (T1)

After 30 days of treatment (T2)
Example of images revised by DERMASURF software: this program allows the evaluation of skin surface through the revision of the DERMATOP BLUE images.
CELLULITE

SLIGHT BLEMISH

PATHOLOGY: Oedematous Fibrocystic Superficial Panniculitis, nodule of degenerate adipose cells surrounded by a sclerotic and scarcely supplied with blood tissue.

A COSMETIC can act as an adjuvant of the treatment against the pathology and as a barrier against the progression of the tissue degeneration in the first phases.
**Cellulite**

- Configuration HE-100
- Filtering and points reduction
- Alignment at the acquisition on live video image
- Removing local shape
- Waviness statistics or roughness for skin
- Surface and volumes of bumps

**Table**

<table>
<thead>
<tr>
<th>Name</th>
<th>S003-Z0-T0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos Vol</td>
<td>82.74 mm³</td>
</tr>
<tr>
<td>Pos Area</td>
<td>630.073 mm²</td>
</tr>
</tbody>
</table>
MAKE UP PRODUCTS

- Eye-liner water resistance test
- Mascara water resistance test
- Lipstick flexibility test
- Lipstick friction strength
HAIR PRODUCTS

Main performance tests on hair products

MORPHOLOGICAL ANALYSIS WITH A SCANNING ELECTRON MICROSCOPY SEM ON HAIR LOCKS AND A FT-IR SPECTROPHOTOMETRY OF THE FORMULATION

Observation of the surface and of hair sections: SEM images for the evaluation, before and after the treatment, of the cosmetic product efficacy, such as shampoo, conditioner, restructuring products, foams, gel...

- Hair dye - determination of the washing resistance: method to evaluate the hair discolouration of hairs treated with dyes. This method is based on the application of the dye on standard tissues and afterward, the measurement of the colour through the measurements CIE L*a*b* (DIN 5033).
SEM PHOTO OF A DEFIBRATED HAIR
(scanning electron microscopy)
DEODORANTS AND ANTIPERSPIRANTS

The skin odour evaluation, in accordance with EEMCO guidelines, is performed through:

**SNIFF TEST, odour evaluation**: the test lasts 3 weeks. The first week is dedicated to conditioning (basal sniff test). In the 2 weeks after follows the application of the product. Two evaluation are performed after 6 and 24 hours from the last product application. The sweat odour intensity is evaluated by a group of 4 judges which applied the following scale:

**Sweat odour intensity (score):**

- Absent 0
- Slightly perceptible 1
- Clearly perceptible 2
- Moderate 3
- Strong 4
- Very strong 5
CONSUMER TEST

COSMETIC EFFICACY

PERCEIVED EFFICACY

MEASURED EFFICACY

Refers to the consumer evaluation and depends on the global impact of the product on the user. The perceived efficacy is the result of the tactile, visual, olfactory and emotional stimulus by the product from the moment of the product choice till the application and observation of the product efficacy on skin.
SENSORIAL ANALYSIS

There are many characteristic which prove the quality of a product but those which determine the customer ACCEPTANCE of a product are the SENSORIAL characteristics, even if it is very difficult to evaluate them with objectivity.

The peculiarity of this analysis is the use of the MAN as evaluation instrument. Even so, this technique allows the “objectification” of the subject thank to the statistical method of the analysis.
SAFETY TESTS

Safety tests have the aim of evaluating the cosmetic formulation compatibility with skin and mucous membrane.

Safety test can be performed:

- **in vitro**
- **in vivo**
In vitro TESTS

In vitro test are performed to analyse new formulations or to verify the product compatibility with mucous membrane and skin (e.g.: products coming from extra EU countries, especially from China)

IN VITRO OCULAR IRRITATION

IN VITRO DERMAL IRRITATION
**In vitro dermal-ocular irritation**

Evaluation of the skin and ocular irritant potential by using, respectively, **Dermal** and **Ocular irritation** tests provided by in vitro-International.

“**Irritection®**” assay consists in a innovative test including the use of specific matrixes and the instrumentation necessary to read and elaborate the obtained data. This is possible thanks to a particular software which allows the determination of the irritating potential of cosmetic products in the different possible shapes.
These tests require the recruitment of volunteers. For this reason it is necessary that a safety evaluator certifies the absence of significant risks for human health (Helsinki Declaration).
TOLERABILITY TESTS

The cosmetic preparations or substances result:
- NOT IRRITATING
- SLIGHTLY IRRITATING
- MODERATELY IRRITATING
- STRONGLY IRRITATING

The most common tolerability tests are: PATCH TEST, OPHTALMOLOGICAL TEST
Ophthalmologic test

This test allows the evaluation of the ocular compatibility with cosmetic formulations destined to come into contact with eyes and the border area.

A useful confirmation of the ocular compatibility of these kinds of cosmetic products is the performance of an *in vivo* test under ophthalmologic control.
Patch test

The aim of the test is to evaluate the skin compatibility of cosmetic formulations destined to come into contact with human skin.

The applied conditions of use (occlusive patch test lasts till 24 hours) have to reproduce the normal or reasonable condition of use of the consumer to assure that the product is not irritant.

DERMATOLOGICALLY TESTED
Results interpretation

The evaluation of skin reactions is performed after 24 and 48 hours of application following the evaluation scale.

<table>
<thead>
<tr>
<th>ERYTHEMA</th>
<th>CLINICAL SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No erythema evidence</td>
<td>0</td>
</tr>
<tr>
<td>Minimal/uncertain erythema</td>
<td>0.5</td>
</tr>
<tr>
<td>Extended, slight scrub redness</td>
<td>1</td>
</tr>
<tr>
<td>Uniform moderate redness</td>
<td>2</td>
</tr>
<tr>
<td>Uniform evident redness</td>
<td>3</td>
</tr>
<tr>
<td>Burning redness</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EDEMA</th>
<th>CLINICAL SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Slight (hardly visible)</td>
<td>1</td>
</tr>
<tr>
<td>Slight (clearly visible)</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>3</td>
</tr>
<tr>
<td>Severe (extend beyond the application area)</td>
<td>4</td>
</tr>
</tbody>
</table>

By calculating the mathematic average of the irritation indexes obtained on the 20 volunteers, it is possible to draw the product classification.
SOLARS

TEST FOR THE EVALUATION OF THE SUN PROTECTION FACTOR

EFFICACY SAFETY
WHAT IS A SOLAR PRODUCT?

A PRODUCT for SUN PROTECTION is: any preparation for “PRODUCT FOR SUN PROTECTION” that means: any preparation (cream, oil, gel, spray) destined to come into contact with human skin, to protect it from UV rays absorbing and dispersing them by refraction.

Law 713/86
PREVENTION AND PROTECTION

SOLAR PRODUCTS can protect - if correctly used - by solar erythema, by photo ageing, and by other skin diseases.
RECOMMENDATIONS OF THE EUROPEAN COMMISSION

The recommendation of the European commission above the efficacy of sun protection products and above the corresponding indications (2006/247/EC) was issued last 22nd September 2006, published on GUUE L265 of 26/09.

It is available on the website of Ministry of Health in the area of cosmetic products.
RECOMMENDATIONS OF THE EUROPEAN COMMISSION

In order to contribute to a high level of wealth safeguard the European Commission retains right to give instructions for a correct use of solar products:

- Correct labelling
- Warnings and precautions for use
- User instructions

- Minimum efficacy: solar products should grant a minimum level of protection from UVB and UVA rays
WHAT ARE THE FEATURES OF SOLAR PRODUCTS?

According to the European commission the characteristics of a solar product are:

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>sun protection factor SPF</th>
<th>measured sun protection factor</th>
<th>recommended Minimum UVA P. factor</th>
<th>minimum Critical recommended wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW PROTECTION</td>
<td>6</td>
<td>6-9.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10-14.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEDIUM PROTECTION</td>
<td>15</td>
<td>15-19.9</td>
<td>1/3 of the SPF indicated on the label</td>
<td>370 nm</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20-24.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25-29.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIGH PROTECTION</td>
<td>30</td>
<td>30-49.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>50-59.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VERY HIGH PROTECTION</td>
<td>50+</td>
<td>60+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SPF - Sun Protection Factor

SPF should be the primary consideration for sunscreen potency

(Consensus position by American Academy of Dermatology, 2001)

Which is the suitable method to measure SPF level?

International SUN PROTECTION FACTOR

COLIPA test method
SUN PRODUCTS: TESTING

TEST : guidelines - protocols

CALCULATIONS: interpretation of the results

QUALITY: criteria of acceptability of results
Initiatives of COLIPA for sun products: TESTING

- **SPF test method** including guidelines for the monitoring of UV source
- Guidelines for the evaluation of water resistance
- Guidelines for the evaluation of UVA protection
- Guidelines for the evaluation of photo stability of UV filters.

In vivo

In vitro
COLIPA initiatives for sun products:

LABELLING

• Recommendations concerning the labelling of sun protection factor (SPF)

• Recommendations concerning the labelling of UVA protection level (UVA)

• Recommendations concerning the use of the sun products (and concerning the sun!!!)
SOLAR SIMULATOR

Definition of solar simulator:
Xenon arc lamp with a filtering system (WG320 + UG11/1mm)
WATER RESISTANCE

This test consists of the comparison of a solar product SPF, calculated after a fixed immersion period in water, with the original (STATIC) SPF, calculated in accordance with the International Sun Protection Factor.

Instrumentation and testing conditions:

1. Spa pool, Jacuzzi or bath tub
2. Water temperature and quality (29 ± 2°C)
3. Standard sequence of the immersions
WATER RESISTANCE: definition for labelling

• WATER RESISTANT:
  2 immersions for 20 minutes
  Efficacy of the remaining SPF > 50%

• VERY WATER RESISTANT:
  4 immersions for 20 minutes
  Efficacy of the remaining SPF > 50%
European Commission recommendation

The recommendation of the European Commission dated 22 September 2006:

- **SPF**: It is the International SPF test Method (even if it is preferable a “future” in vitro method)
- **UVA protection**: it prefers the in vitro test with a UVA protection equal to 1/3 of the SPF protection.
- **It take into consideration the PHOTODEGRADABILITY of the UV filters**
UVA protection
Which is the method?

IN VITRO

UVA RATIO - BOOTS STAR RATING SYSTEM

recommended CRITIC WAVELENGTH > 370 nm

<table>
<thead>
<tr>
<th>UVA RATIO</th>
<th>STAR CATEGORY</th>
<th>STAR RATING</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 a 0.2</td>
<td>_</td>
<td>Too Low for UVA claim</td>
</tr>
<tr>
<td>0.2 a 0.4</td>
<td>*</td>
<td>MODERATE</td>
</tr>
<tr>
<td>0.4 a 0.6</td>
<td>**</td>
<td>GOOD</td>
</tr>
<tr>
<td>0.6 a 0.8</td>
<td>***</td>
<td>SUPERIOR</td>
</tr>
<tr>
<td>≥ 0.8</td>
<td>****</td>
<td>MAXIMUM</td>
</tr>
</tbody>
</table>
in VITRO UVA Test

June 2009

• METHOD FOR THE IN VITRO DETERMINATION OF UVA PROTECTION AND “CRITICAL WAVELENGTH” VALUES OF SUNSCREEN PRODUCTS

• (Prepared by the COLIPA in vitro Photoprotection methods Task Force)

VALIDATED IN COMPARISON WITH THE IN VIVO METHOD
Importance of the in vitro UVA determination

- Validated on the basis of the in vivo method results (Persistent Pigment Darkening PPD method)
- The measurements are the basis for a uniform claim: it will be easier for the Consumer to choose the sun product with UVA protection
- The European Commission prefers the in vitro tests
- It is easily reproducible from different laboratories
- It is faithful to the using conditions and to the Consumers expectations
- It is free from ethic implications: no “human subjects“ are involved!
Method principle

This test is based on the evaluation of the transmittance by using a solar thin layer distributed on a special substratum, before and after the exposure to a certain dose of radiations coming from a specific UV source.
1st example of ABSORBING CURVE before and after the UV irradiation
2nd example of ABSORBING CURVE before and after the UV irradiation

Before UV exposure

After UV exposure
Labelling of UVA protection

In accordance with the Colipa Recommendation
If the UVA protection is equal or superior to the relation $1/3$ the solar can be labelled with:

UVA
Microbiological safety

Together with performance tests also the microbiological controls are very important to evaluate a product safety.

<table>
<thead>
<tr>
<th>PRODUCT TYPOLOGY</th>
<th>SUSCEPTIBILITY TO THE MICROBIAL DEVELOPMENT</th>
<th>CONTROL OF THE MICROBIAL ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{st}) Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emulsions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lotions and aqueous solutions</td>
<td>High</td>
<td>The microbial development is controlled with preservatives and in accordance with good manufacturing practices</td>
</tr>
<tr>
<td>Tensiolites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous gel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pastes according to the formulation</td>
<td>High</td>
<td>The microbial development is controlled with preservatives and in accordance with good manufacturing practices</td>
</tr>
<tr>
<td>2(^{nd}) Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pastes and gel according to the formulation</td>
<td>Medium</td>
<td>The microbial control is carried out by using preservatives, other ingredients and it also depends on the formulation: pH, water activity, antimicrobials specific for a certain product (deodorants).</td>
</tr>
<tr>
<td>Lotions, emulsions, foams according to the pH level and/or other ingredients</td>
<td>Medium</td>
<td>The microbial control is carried out by using preservatives, other ingredients and it also depends on the formulation: pH, water activity, antimicrobials specific for a certain product (deodorants).</td>
</tr>
<tr>
<td>Powder products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3(^{rd}) Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidizing and reducing products</td>
<td>Law</td>
<td>In these products micro-organisms can survive without multiplying their number.</td>
</tr>
<tr>
<td>Alcoholic products and products based on other solvents</td>
<td>Law</td>
<td>In these products micro-organisms can survive without multiplying their number.</td>
</tr>
<tr>
<td>Oleolites and lipogels</td>
<td>Law</td>
<td>In these products micro-organisms can survive without multiplying their number.</td>
</tr>
<tr>
<td>Anhydrous pastes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipidic fusions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not aqueous solutions</td>
<td>Law</td>
<td>In these products micro-organisms can survive without multiplying their number.</td>
</tr>
</tbody>
</table>
Acceptability levels of the microbial activity in cosmetic products

<table>
<thead>
<tr>
<th>PRODUCTS CATEGORY</th>
<th>MESOPHILIC AEROBIC GERMS</th>
<th>SPECIFIC MICROORGANISMS (*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1 – baby products (Age &lt; 3 years) – for periocular use</td>
<td>100 UFC / gr or ml</td>
<td>Absent in 1 g or ml</td>
</tr>
<tr>
<td>Category 2 – Other products</td>
<td>1000 UFC / gr or ml</td>
<td>Absent in 1 g or ml</td>
</tr>
</tbody>
</table>

(*) Specific micro-organisms has to be detected on the sample unit:
Pseudomonas aeruginosa, Staphilococcus aureus, Candida albicans, Escherichia coli

The updated methods for the microbial analysis on cosmetic products have been issued by ISO
Methods for the microbial analysis

Chelab carries out microbiological analysis according to the following ISO standard methods:

-Mesophilic Aerobic Bacterial Count (ISO 21149:2006)

This determination constitutes a rapid, effective and economic control that should be routinierly used to verify the microbiological quality of the cosmetic formulations.

Further to this verification it is suggested to perform also the mycetes determination to complete the basic microbiological control of the cosmetics.

From these initial verifications, it could be even necessary to procede with the research and the identification of possible pathogenic microorganisms, that can constitute a danger for the consumers’ health.
Methods for the microbial analysis

-Myctes (ISO 16212:2008)

In microbiology, the term “myctes” generally indicates the moulds and the yeasts, which are microorganisms that can compromise the cosmetic product quality and can represent a possible cause of risk for the consumers.

For this reason, together with the determination of the bacteria count, the verification of the presence of these microorganisms in the cosmetic products constitutes a rapid, effective and economic control.
Methods for the microbial analysis

- *Staphylococcus aureus* (ISO 22718:2006)
- *Escherichia coli* (ISO 21150:2006)

The verification of the presence of this pathogenic strains in cosmetic products follows these phases: enrichment phase in a non-selective neutralizing media and then isolation of the specific strain in a suitable soil.

If there is the growth of characteristic colonies on the selective soil, other tests are performed to confirm the identity of the microorganism that has created the observed colonies.
A cosmetic product has to contain preservatives in order to avoid the microbial contamination. The anti-microbial efficacy has to be tested by the formulator of the producer with CHALLENGE TEST.

This test consist in the artificial contamination of the finished cosmetic product in order to evaluate the efficacy of its preservative system.

- Aspergillus brasiliensis
- Candida albicans
- Escherichia coli
- Pseudomonas aeruginosa
- Staphylococcus aureus

Microorganisms
Challenge test

Internal method (MP 1033, accredited by ACCREDIA)

The micro-organisms commonly studied are: Aspergillus brasiliensis, Candida albicans, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. It is however possible to employ different micro-organisms, even of wild-type strains, based on the customer's needs. The test consists in the inoculation at known titration, of microorganisms into the product and, through their survival time analysis, in the determination of the preservative system efficacy.
Challenge test

Internal method (MP 1033, accredited by ACCREDIA)

The determination of the Units Forming Colonies per gram (UFC/g) is performed when inoculating the organism (Time zero) and at the following times:

<table>
<thead>
<tr>
<th>Logarithmic reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRITERIA</td>
</tr>
<tr>
<td>Bacteria A</td>
</tr>
<tr>
<td>Bacteria B</td>
</tr>
<tr>
<td>Fungi A</td>
</tr>
<tr>
<td>Fungi B</td>
</tr>
</tbody>
</table>
Challenge test

Afnor method

The determination of the Units Forming Colonies per gram (UFC/g) is performed when inoculating the organism (Time zero) and at the following times:

<table>
<thead>
<tr>
<th></th>
<th>Bacteria</th>
<th>Yeasts</th>
<th>Moulds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
<td>14 days</td>
<td>28 days</td>
</tr>
<tr>
<td>Profile A</td>
<td>≥ 3 log</td>
<td>No increase</td>
<td>No increase</td>
</tr>
<tr>
<td>Profile B</td>
<td>≥ 3 log</td>
<td>No increase</td>
<td>No increase</td>
</tr>
<tr>
<td>Profile C</td>
<td></td>
<td></td>
<td>Case by case definition</td>
</tr>
</tbody>
</table>

The aim of these criteria is to evaluate the antimicrobial effectiveness of a cosmetic product. The criteria for profile A and B are defined. For the formulation with a profile C, it is up to the producer to define and justify the applicable criteria, considering the evaluation of the microbiological risk.

Note: Two results can be considered different only if their difference exceeds 50%, or, express in log, only if exceeds 0.3 log.
Challenge test

CTFA criteria

Criteria for evaluation of antimicrobial-effectiveness according to cosmetic, toiletry, and fragrance association (CTFA).

- **BACTERIA**: after 7 days the reduction must be at least $\geq 3$ logarithms and must not increase until the end of the test

- **YEASTS and MOULDS**: after 7 days the reduction must be at least $\geq 1$ logarithm and must not increase until the end of the test
Why perform efficacy tests?

The correct and objective performance of these tests confers SCIENTIFIC (and ECONOMIC) value to the cosmetic product.

Why perform safety tests?

Safety tests are necessary to assess that a certain product is riskless for the consumers if used in the normal conditions of use.
BIOCIDES

CHELAB SERVICES ON BIOCIDAL PRODUCTS
Product types

- **MAIN GROUP 1: Disinfectants and general biocidal products**
  - Product-type 1: Human hygiene biocidal products
  - Product-type 2: Private area and public health area disinfectants and other biocidal products
  - Product-type 3: Veterinary hygiene biocidal products
  - Product-type 4: Food and feed area disinfectants
  - Product-type 5: Drinking water disinfectants

- **MAIN GROUP 2: Preservatives**
  - Product-type 6: In-can preservatives
  - Product-type 7: Film preservatives
  - Product-type 8: Wood preservatives
  - Product-type 9: Fibre, leather, rubber and polymerised materials preservatives
  - Product-type 10: Masonry preservatives
  - Product-type 11: Preservatives for liquid-cooling and processing systems
  - Product-type 12: Slimicides
  - Product-type 13: Metalworking-fluid preservatives
Product types (2)

• MAIN GROUP 3: Pest control
  • Product-type 14: Rodenticides
  • Product-type 15: Avicides
  • Product-type 16: Molluscicides
  • Product-type 17: Piscicides
  • Product-type 18: Insecticides, acaricides and products to control other arthropods
  • Product-type 19: Repellents and attractants

• MAIN GROUP 4: Other biocidal products
  • Product-type 20: Preservatives for food or feedstocks
  • Product-type 21: Antifouling products
  • Product-type 22: Embalming and taxidermist fluids
  • Product-type 23: Control of other vertebrates
Key regulatory documents

- Directive 98/8 CE
  Contains the details of the regulation and the updated list of active substances that are completely under this regulation

- Regulation 1451/2001
  Work program for the evaluation of the dossier of the active principle listed in annex II and sponsored by a company
Turkey acknowledged the regulation
OVERVIEW OF CHELAB BIOCIDAL SERVICES

Chelab provides a full spectrum of analytical testing and advisory services with regard to:

- development and validation of methods for identification and analysis
- GLP and OECD Technical Guideline conforming studies:
  - physical, chemical and technical properties of the product
  - stability studies
  - in vitro toxicological studies
  - ecotoxicological studies
  - biocidal efficacy assessment
- risk assessment for:
  - human health
  - fate and behaviour in the environment
- active substance dossier preparation and submission for the inclusion in annexes I, IA and IB
- product dossier preparation and submission for the authorization and registration of products
Thanks for your kind attention!
İlginiz için teşekkürler!

Chelab srl