



MICROBIOLOGICAL SAFETY OF TOY

INTRODUCTION AND SCOPE

The scope of the testing procedure is to evaluate the presence of a microbiological contamination in toys.

The presence of pathogens micro-organism can result in a serious risk for the children in using the toy.

In the absence of a specific normative that regulate the safety of toys from a microbiological point of view, we have referred, in order to define the test to perform and the safe limit to apply, to cosmetic requirements.

A microbiological evaluation will reduce risks related to a contamination from bacteria due to the following exposure routes:

- skin contact
- eye contact
- ingestion, also

The individuation of the field of application has been done considering that the proliferation of micro-organism is connected with the presence of water, therefore the risk associated with the use of liquid containing toy and toys which use is strictly related to water is the big one.

Cosmetic are not included in the scope of this evaluation since they follows their own specific rules.

FIELD OF APPLICATION

- 1. Accessible liquid containing water** as defined in EN 71-9, accessible liquid is a liquid in or on a toy or accompanying a toy which the child is likely to become exposed during normal or foreseeable use of the toy (e.g. liquid paints, ink in pens, liquid provided with toys for squirting)
- 2. Modelling clay, play clay and similar**
- 3. finger paints**

LIMIT

The sample can be considered safe if:

Flora Mesofila Aerobia Aerobic Mesofile Flora	≤ 100 ufc/g
Lieviti e muffe leaven and mould	≤ 10 ufc/g
Staphylococcus aureus	Absent
Pseudomonas spp	Absent
Candida albicans	Absent
Gram negative Bacteria	Absent
Obligate or facultative anaerobic bacteria	Absent

AEROBIC MESOFILE FLORA, LEAVEN AND MOULD

Bibliographic references for aerobic mesofila flora, leaven and mould with special attentino to Staphylococcus aureus, Pseudomonas spp., Candida albicans, Bacilli Gram negativi coltivabili su Mac Conkey Agar:

- European Pharmacopeia, 4th editino, 2002 (“microbiological examination of non-sterile products”)
- CTFA Technical Guidelines, G.N. McEwen Jr. and A.S. Curry, Eds., Washington DC, 2001
- XXV U.S.P. (microbiological test: “Microbial Limit Tests”)
- Manual of Clinical Microbiology, Murray P.R. et al., American Society of Microbiology, 7th edition, ASM Press, 1999

Dilution used 1/10, obtained dispersing some sample in Lethen-Broth Base modified. If necessary also diluition 1:100 and 1:1000 could be prepared.

For Aerobic Mesofile Flora, in plate: Caso Agar, incubation 32 ±2°C for 72±6 hours.
For leaven and mould, in plate: Potato-dextrose agar, incubation 25 ±2°C for 72±6 hours.

Research of Staphylococcus aureus, Pseudomonas spp., Candida albicans, Bacilli Gram-negative is done using Lethen-Broth Base Modified, incubation 32±2 °C for 24±2 hours. The specific procedures for each group of bacteria are:

- Staphylococcus aureus: Baird-Parker agar, incubation 32 ±2°C for 48±3 hours. Performed Gram colouring, catalase test and Staphylase test. It is considered Staphylococcus aureus each colony positive to Gram colouring, to catalase test and to rapid hemagglutination on slide;
- Pseudomonas spp: Cetrimide Agar, incubation 32 °C for 48±3 hours. Performed Gram colouring and oxidase test to confirm Pseudomonas spp., it should come out Gram-negative and oxidase positive;
- Candida albicans: Sabouraud Gentamicina cloramfenicolo, incubation 25 ±2°C for 48±3 hours. It is considered Candida albicans each colony forming germinative tubes;
- Bacilli Gram negativi: Mac CanKey Agar, incubation 32°C for 48±3 hours. Performed Gram colouring and oxidase test to exclude Pseudomonas presence.

Results are expressed in unity forming colony (ufc) for grams of products (cfu/g).

OBBLIGATE AND FACULTATIVE ANAEROBIC BACTERIA

Bibliographic references for obligate or facultative anaerobic bacteria:

- Official Pharmacopeia, X Edition (vol.1 “microbiological examination of non-sterile products”)
- XXIV U.S.P. (microbiological test: “Microbial Limit Tests”)
- Notes of guidance for testing of cosmetic ingredients for their safety evaluation, Revision of Annex 7: Microbiological Quality of Finished Cosmetic Product adopted by the plenary session of the SCCNFP of 23 September 1998
- Manual of Clinical Microbiology, Murray P.R. et al., American Society of Microbiolog, 7th edition, ASM Press, 1999.

Dilution used 1/10, obtained dispersing some sample in Letheen-Broth Base modified. Thioglycollate Broth, incubation 32 ±2°C for 48±3 hours.

A part of this solution is posed on CNA agar and Caso agar, incubation 37±2°C for 48 ±3 hours, and on Patato dextrose agar, incubation 25±2°C for 48 hours.

We consider to have a positive result in case of growth only on CNA agar.