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COSMETIKIT ®-ISO

100% COMPLIANT WITH ISO STANDARDS ON COSMETIC MICROBIOLOGY

INTRODUCTION

Reference: KMT460.

With the simple help of a water bath and culture oven, any cosmetics company can use this kit to perform full and official (ISO cosmetic standards) microbiological analyses of 20 different samples. For optimal results, please use our COSMETIKIT-CLASSIC (ref: KMT444).

If you also wish to test water, please use our COSMETIKIT-WATER (ref: KMT450). For work surfaces, recipients etc., use DESINFECTEST® culture slides (E.g. ref: MBN407) and for operators' hands, our HANDLERS' KITS ref: KMT020).

CONTENTS (SHELF LIFE APPROX. 1 YEAR FROM DATE OF MANUFACTURE)

*20 sterile 20ml syringes (needleless).
*20 sterile Pasteur pipettes.
*2 x 10 bottles 90ml w/beads to treat the sample (Eugon LT100 Broth ISO 21148, ref: RPL026P).
*20 tubes for total count (TSA ISO 21149, ref: TPL077).
*20 tubes for yeasts and moulds (SDA Chloramphenicol. ISO 16212 ref: TPL073).
*20 tubes for *Pseudomonas aeruginosa* (Cetrimide Agar ISO 22717 ref: TPL100).
*20 tubes for *E.coli* and Coliforms (EMB Agar ISO 21150 ref: TPL088).
*20 tubes for *Staphylococcus aureus* (Mannitol Salt Agar ISO 22718 ref: TPL066).
*20 slanted tubes for *Candida albicans* (Biggy Agar ISO 18416 ref: TPL062).
*20 tubes for *Burkholderia cepacia* (BCPT Agar ISO 22717 ref: TPL005).
*120 sterile Petri dishes 90 mm.

EQUIPMENT REQUIRED (NOT INCLUDED)

- 35-37ºC oven (VRP001), ➢Water bath, boiling water bath or microwave,
- Aseptic area: Spirit burner (VLM068) or Portabunsen (ME2195+ME2196) and Envirosteril (VJM002) if no laminar flow cabinet available.
- Tests to confirm suspect colonies (all available from MICROKIT).
- Reference, work or quantitative strains to validate reagents once delivered to the site or after prolonged or inappropriate storage (See MICROKIT stable quantitative lenses)
- Participate in intercomparative services, such as SEILA-PARFUM to validate processes and analyses.

INSTRUCTIONS FOR USE (Follow closely to obtain correct, valid results)

1.- Aseptically, using a sterile syringe, add 10g or 10ml of sample to a 90ml Eugon LT100 Broth bottle with beads. Close the cap. Shake to mix and leave for between 20 and 30 minutes at approximately 21-

25°C to obtain the stock solution (treated sample).
2.- Using a sterile Pasteur pipette, immediately add 1ml of the recently mixed treated sample to a sterile Petri dish under aseptic conditions. Repeat the operation on another dish, using the same sample and the same pipette. It is advisable to create duplicates (Order TPL077 and TPL073).
3.- Using the water bath, melt a tube of TSA and another of SDA Chloramphenicol until completely liquid (if duplicating the plates, melt two tubes of each medium per sample).
4.- When it has sufficiently cooled so as not to burn your hand, but remaining liquid, add each to a plate with the millilitre of treated sample. Moulds may also be surface plated, 0.1-0.33ml using a Drigalski spatula. It is advisable to mass plate 1ml and surface plate 0.1ml.
5.- Swirl the plates on the table 10 times in each direction to mix the medium with the sample, ensuring that the liquid does not overspill or reach the lid of the plate. Leave, without touching, to solidify (if the ambient temperature is not too high, 10-15 minutes will suffice).
6.- Incubate the plates upside down in complete darkness for 3-5 days at 30-35ºC (TSA) and (3)-5 days at 20-25ºC (SDA Chloramphenicol).
7.- At the same time as these plates, incubate the rest of the treated sample in Eugon LT100 Broth for 48 hours at 30-35°C to obtain the enriched treated sample required to search for pathogens. It is also possible to put 10ml in a TSB bottle (order ref: RPL043) and 10ml in a Lactose Broth LB bottle (order ref: RL017) and incubate, but the absence of pathogens will then only be in 1g, rather than in 10g.
8.- Add some drops (after enrichment, obviously) of the enriched sample, recently shaken, to a slanted Biggy Agar Candida tube, using the same type of sterile pipette and spread, turning.
9.- Add some drops of the enriched treated sample, recently shaken, to a sterile Petri dish under aseptic conditions, for Mannitol Salt Agar. If enriched in TSB and LB, use the TSB to inoculate all the plates, except EMB Levine Agar, which is inoculated using the LB.
10.- Using the water bath, melt a tube of EMB Levine Agar, another of Mannitol Salt Agar, another of Cetrimide Agar and another of BCPT Agar until completely liquid.
11.- When it has sufficiently cooled so as not to burn your hand, but remaining liquid, add the contents of Mannitol Salt Agar to the plate with the drops of enriched sample.
12.- Swirl the plates on the table 10 times in each direction to mix the medium with the sample, ensuring that the liquid does not overspill or reach the lid of the plate. Leave, without touching, to solidify. This mass plating is indispensable for staphylococci.
13.- Pour the contents of the EMB, Cetrimide and BCPT on two separate sterile Petri dishes and leave to solidify. Spread a drop of the recently shaken enriched sample in a zigzag pattern over the surface of each.
14.- It is good practice to also place a drop of the recently shaken enriched sample on another TSA plate to isolate and identify colonies (but not to count them!), in order to increase sensitivity for stressed strains that may not grow in selective medium. Order additional TPL077 tubes.
15.- Incubate the plates upside down and the tubes of *Candida albicans* in a vertical position for 24-72 hours at 30-35ºC. Do not stack more than 5 plates and leave space between the stacks, as well as space between the stacks and the walls of the oven.

INTERPRETATION OF RESULTS

Total count (TSA plates) should not exceed 100///1000 ufc/ml or gram of initial sample, depending on requirements (infant cosmetics and generally for suppressed immune systems). This means that there should not be more than 10-100 colonies per plate on account of the 1/10 dilution of the stock solution. The same applies for yeast counts (convex colonies) and mould counts (filamentous colonies) (SDA Chloramphenicol plates). Otherwise, provided that there are no pathogens, the batch may be reprocessed.

No colonies of *Escherichia coli* should be apparent (metallic colonies in EMB plates. The other colonies in this medium are indicators of coliforms, which, without being pathogenic or exclusive indicators of faecal contamination, generally alter the sample), *Pseudomonas aeruginosa* (yellow-green colonies or reddish-brown colonies in Cetrimide plates), *Burkholderia cepacia* (white or salmon coloured colonies, with medium turning to fuchsia in BCPT), or *Staphylococcus aureus* (yellow colonies in Mannitol plates), which are pathogens preceding from faecal, water, water biofilm and air-operator contamination respectively.

If a brown colony appears (that does not cause the medium to turn to brownish-black) in the Biggy tube, this will be *Candida albicans*, demonstrating contamination by mucus from operators. If any of the 5 pathogens appear, the batch must be destroyed.

For final confirmation, please ask for details of our suspect colonies identification kits.