MICROBIAL CONTENT TEST AGAR
(TSA+LECITHIN+TWEEN® 80)
Dehydrated culture medium

1 - INTENDED USE
General purpose medium for the detection and enumeration of microorganisms on treated sanitary areas, containers, equipment.

2 - COMPOSITION -TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of casein	15.0 g
Soy peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Lecithin	0.7 g
Polysorbate 80	5.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Detection and enumeration of microorganisms on environmental surfaces treated with antiseptics have been the subject of many investigations by microbiologists in the public health field. Quisno *et al*[1] reported that lecithin would effectively neutralizes quaternary ammonium compounds and Brummer[2] combined lecithin and polysorbate 80 for the neutralisation of disinfectants by replicate organism detection and counting (RODAC) technique.
Microbial Content Test Agar, prepared with Tryptic Soy Agar supplemented with lecithin and polysorbate 80, is used for the detection and enumeration of microorganisms surviving after treatment of surfaces and materials with antiseptics and in environmental air sampling procedures.
Tryptic Soy Agar is the medium specified as “casein soya bean digest agar” in the harmonised EP, USP JP method[3] for microbial enumeration of non-sterile pharmaceutical products. Lecithin is incorporated to neutralize quaternary ammonium compounds and polysorbate 80 is used to neutralize substituted phenolic disinfectants.

4- DIRECTIONS FOR MEDIUM PREPARATION
Suspend 45.7 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C mix well and pour into sterile Petri dishes or RODAC plates.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	pale yellow, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, opalescent
Final pH at 20-25 °C	7.3 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Microbial Content Test Agar	Dehydrated medium	4016992	500 g (10.9 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED
Autoclave, water-bath, sterile loops, needles, swabs, sponges, incubator and laboratory equipment as required, Petri dishes, RODAC plates, Erlenmeyer flasks, ancillary culture media and reagents.

8 - SPECIMENS
Sanitary areas, containers, equipment treated with disinfectants.

9 - TEST PROCEDURE
RODAC plates filled with Microbial Content Test Agar are employed for the quantitative method to enumerate microbes on open flat work surfaces.[4]
- Gently but firmly touch the RODAC agar surface against the area being sampled, exert moderate, even, vertical pressure and then carefully replace lid. Avoid using rubbing motions of the plate at the sample site as this may break the agar.
- Incubate exposed plates at 35-37°C for 48 hours, and 25°C for 7 days or as required.

Qualitative methods utilizing sponges/ swabs are used for hard-to-reach areas.[4]
- Apply swab (or sponge with handle) to surface (or equipment) being monitored with firm application pressure.
- When sampling (monitoring) flat surfaces allow the swab (or sponge with handle) to firmly rub an area of approximately 24 to 30 cm^2.
- Apply the swab (or sponge) within this contact area in both a horizontal and vertical direction for approximately 10 seconds.
- Roll the swab directly on the surface of Microbial Content Test Agar plates.
- Incubate inoculated plates at 35-37°C for 48 hours, and 25°C for 7 days or as required.

10 - READING AND INTERPRETATION
After incubation, the presence of microorganisms is indicated by the appearance of colonies of various morphology and size on medium surface. The characteristics of the growth are closely related to the type or types of cultivated microorganisms.
Count all developed colonies.

11 - USER QUALITY CONTROL
All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control of un-supplemented medium.

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CONTROL STRAINS			INCUBATION T°/ t / ATM	EXPECTED RESULTS
S. aureus	ATCC	6538	35-37°C / 18-24H / A	good growth
E. coli	ATCC	25922	35-37°C / 18-24H / A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Microbial Content Test Agar is tested for productivity and neutralising properties of a quaternary ammonium base by comparing the results with a previously approved Reference Batch (RB) and with a general-purpose medium without neutralising properties (Tryptic Soy Agar).
The above characteristics are tested with the following strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 6538.
After incubation at 37°C for 24 hours, good growth of the tested organisms is obtained with un-supplemented Microbial Content Test Agar and supplemented with quaternary ammonium base, whereas the growth is totally or partially inhibited in Tryptic Soy Agar supplemented in the same way.

13 - LIMITATIONS OF THE METHOD

- The effectiveness of preservative neutralization with this medium depends on both the type and concentration of the preservative(s).

14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the preparation process of media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the period of validity of the finished products, according to the type (tubes/bottles), and the storage method applied (temperature and packaging). According to MacFaddin the self-prepared medium may be stored at 2-8°C for 6-8 weeks.[5]

16 - REFERENCES

1. Quisno R, Gibby IW, Foter MJ. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am J Pharm 1946; 118:320.
2. Brummer B. Influence of Possible Disinfectant Transfer on Staphylococcus aureus Plate Counts After Agar Contact Sampling. App Environ Microbiol 1976; 32:80-84.
3. European Pharmacopoeia 10.3, 2021.
4. Food and Drug Administration. Office of Regulatory Affairs. Office of Regulatory Science. Pharmaceutical Microbiology Manual. Document n° ORA.007. Rev 2. 25 Aug 2020.
5. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/09

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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