Mannitol Salt agar medium (IV)

355-3647 / 355-3648
356-3844 / 356-3926
356-4134

DEFINITION
Medium used for the detection and enumeration of *Micrococcaceae* (including pathogenic staphylococci) in the testing of water in swimming pools.
Equivalent USP 30/NF 25: Medium IV

STANDARDS
- **USP 30/NF 25 US Pharmacopeia and National Formulary (2007):** Microbial Limit Tests (61) - Microbiological Tests.

PRINCIPLE
The principle of the medium relies on the ability of most pathogenic staphylococci and *Micrococcaceae* to ferment mannitol (yellow halo around colonies due to change in the indicator color).
Due to the high concentration of sodium chloride, the medium inhibits with regard to other bacteria.

PRESENTATION
- **Pre-poured**
  20 plates x 90 mm — **code 356-3844**
  10 plates x 55 mm — **code 356-3926**
- **Ready to use**
  200 ml x 6 bottles — **code 355-3647**
  10 ml x 25 tubes — **code 355-3648**
- **Dehydrated**
  500 g — **code 356-4134**

STORAGE
- Pre-poured: + 2°to 20℃.
- Ready to use: + 2°to 8℃.
- Dehydrated: + 15°to 25℃, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

TYPICAL FORMULA

Bacteriological peptone	10 g
Bovine meat extract	1 g
Sodium chloride	75 g
Mannitol	10 g
Phenol Red	25 mg
Agar	15 g
Distilled water	1,000 ml

Final pH (25℃) final = 7.5 ± 0.2

Remark: the formula has been adapted to attain the required performance criteria.

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- Diluent(s)
- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- 200 ml Pyrex bottles with autoclave-proof stoppers
- Sterile Petri dishes (Ø = 90 and Ø = 55 mm)
- Filtration apparatus
- Filter membranes (Ø = 47 mm and ≤ 0.45 mm)
- Tweezers for handling membranes
- Water-bath precise to ± 1℃
- Thermostatically-controlled incubator or incubation room, precise to ± 1℃
- Autoclave
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM
Always shake well before use
Dissolve 111 g of powder in 1 liter of distilled water, mix until a homogenous suspension is obtained.

Heat gently, swirling frequently, then bring to boiling point until completely dissolved.
Dispense 200 ml per bottle or 20 ml per tube and sterilize in autoclave at 121℃ ± 1℃ for 15 minutes.
Pour into Petri dishes and leave to dry.

Reconstitution ratio: 111 g/l.
500 g of powder makes 4.5 liters of medium.

PROTOCOL
- **Preparation of samples**

According to the standards or recommendations applicable the product concerned.
- **Inoculation and incubation**

Membrane-filtration method:
Filter 100 ml of sample and deposit the membrane on the surface of a previously filled and dried Petri dish.
Surface inoculation method:
Inoculate 0.1 ml of sample to be analyzed, or its decimal solutions, and spread.

Bio-Rad, 3 boulevard Raymond Poincaré • 92430 Marnes-la-Coquette
Tel. : +33 (0)1 47 95 60 00 • Fax : +33 (0)1 47 41 91 33
www.foodscience.bio-rad.fr

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In both cases, incubate at 37℃ ± 1℃ for 48 hours.

READING AND INTERPRETATION

Enumerate the different types of white or yellow colonies surrounded by a yellow halo, eliminating the large mucous colonies corresponding to bacteria of the *Bacillus* genus.

For each type of colony, take a sample and carry out Gram-staining: colonies formed by Gram positive cocci can be considered *Micrococcaceae*.

To enumerate pathogenic staphylococci, collect a colony of each type (previously identified as *Micrococcaceae*) and sub-culture in brain-heart broth **(code 355-3664)**. After 24 hours incubation at 37℃, carry out a coagulase test on rabbit plasma **(code 355-6352)**.
Consider as pathogenic staphylococci any colonies identified as *Micrococcaceae* which also possess coagulase for rabbit plasma.

PRECAUTIONS

- Comply with Good Laboratory Practice.

QUALITY CONTROL

In view of the current harmonization of pharmacopeias, we recommend that you refer to the certificates of analysis for procedures relating to the quality control (performance and selectivity) of media produced by Bio-Rad.

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

Chapman mannitol/*Micrococcaceae*/ Pathogenic staphylococci/Swimming pool water/Detection/Enumeration/Mannitol/ Medium.

BIBLIOGRAPHY

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