SEMI-SOLID MODIFIED SCHOLTENS' AGAR BASE (ssMSA)
CALCIUM CHLORIDE SOLUTION
NALIDIXIC ACID SELECTIVE SUPPLEMENT

Dehydrated culture medium, supplements and ready to use flasks

1 - INTENDED USE

Semi-solid medium, in accordance with ISO 10705-2, for the enumeration of somatic coliphages in water.

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

Peptone	10.00 g
Yeast extract	3.00 g
Meat extract	12.00 g
Sodium chloride	3.00 g
Sodium carbonate	0.75 g
Magnesium chloride ($6H_2O$)	0.60 g
Agar	6.65 g

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

CALCIUM CHLORIDE SOLUTION (1M)
LIQUID SUPPLEMENT– CONTENT: 30 mL

Calcium Chloride	3.3 g
Water	30 mL

NALIDIXIC ACID SELECTIVE SUPPLEMENT
FREEZE-DRIED SUPPLEMENT – CONTENT: 1 VIAL

Nalidixic acid	125 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Semi-Solid Modified Scholtens' Agar Base (ssMSA) is prepared in accordance with the formulation proposed by the ISO 10705-2[1] standard for the enumeration of somatic coliphages in water. Somatic coliphages are viruses that belong to the large family of bacteriophages: these are capable of selectively infecting bacteria to replicate, injecting their DNA inside the bacterial cell. Subsequently, the virus begins to multiply inside the bacterium causing its lysis, which will be visible in the culture medium (plaque). Somatic coliphages are indicators of faecal pollution in water, because of their ability to specifically attack the *Escherichia coli* species.
ss Modified Scholten's Agar is a semi-solid medium used for host strain dissemination. Peptone, yeast extract and meat extract provide the necessary nutrients for *Escherichia coli* (host), sodium chloride, magnesium chloride and calcium chloride maintain osmotic balance without interfering in phage propagation. The reduced quantity of agar allows mixing of the reference culture with the sample and the diffusion into the base plate (Modified Scholtens' Agar).

4A- DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 36 g in 1000 mL of cold purified water. Mix thoroughly and warm to completely dissolve the powder and sterilize by autoclaving at 121°C for 15 minutes. Cool at 45-50°C and add 6 mL of Calcium Chloride Solution (REF 421020) and, in case of suspected water with high contaminating flora, add 2 vials of Nalidixic Acid Selective Supplement (REF 4240067), reconstituted with 5 mL of sterile purified water, to obtain a final concentration of 250 mg/mL

4B- DIRECTIONS FOR PREPARATION OF READY TO USE FLASKS

Dissolve the contents of the bottle in an autoclave at 100 ± 2°C or in a temperature-controlled water bath at 100°C. Alternatively, the bottle can be placed in a container containing water, which is placed on a heating plate and brought to the boil; loosen the cap slightly before heating. Cool the medium to 45-50°C and add Calcium Chloride Solution (0.6 mL per 100 mL of final medium), included in the package. Distribute in sterile plates with aseptic precautions. In case of suspected water with high contaminating flora, add 1 mL of Nalidixic Acid Selective Supplement (REF 4240067), reconstituted with 5 mL of sterile purified water, to obtain a final concentration of 250 mg/mL. Distribute in sterile plates with aseptic precautions.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	yellow, fine, homogeneous, free-flowing powder
Prepared tubes appearance	beige, limpid
Calcium Chloride Solution Appearance	colourless, clear
Nalidixic Acid Selective Supplement Appearance	Compact white tablet, (clear and colourless solution after reconstitution)
Final pH of complete media (at 20-25°C)	7.2 ± 0.5

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
semi-solid Modified Scholtens' Agar Base (ssMSA)	Dehydrated medium	4017492 4017494	500 g (13.8 L) 5 kg (138 L)
semi-solid Modified Scholtens' Agar Base (ssMSA)	Kit: Ready to use flasks+ Calcium Chloride Solution	511749K2 511749K3	6 flasks, each for 100 mL of medium + 1 vial (4 mL) of Calcium Chloride Sol. 1M 6 flasks, each for 200 mL of medium + 2 vials (4 mL) of Calcium Chloride Sol. 1M
Calcium Chloride Solution	Liquid supplement	421020	30 mL
Nalidixic Acid Selective Supplement	Freeze-dried Supplement	4240067	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, sterile Petri plates, ancillary culture media and reagents.

8 - SPECIMENS

This method, taken from the ISO 10705-2 standard, is suitable for all types of water (natural or waste) and sludge. For collection, transport, storage of samples and culture of the host strain, follow good laboratory practices and refer to the applicable standards (ISO 8199, ISO 5667-1 and ISO 5667-3)[2-4].

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9 - TEST PROCEDURE

Standard procedure for setting up the test

For the isolation of somatic coliphages and plaque counting, in accordance with ISO 10705-2, the following method is recommended:

1. Prepare the host strain as required by the reference standard.
2. Prepare Modified Scholtens's Agar plates (REF 401748) as described.
3. Prepare 50 mL of ssMSA as described above (in case of water with high quantity of pollutants, add Nalidixic Acid Solution REF 4240067 to obtain a final concentration of 250mg/L)
4. Prepare various dilutions of the water to be treated and dispense 1mL into test tubes for each dilution (it is recommended to prepare 2 test tubes for each dilution to make the plaque count more accurate)
5. Dispense the dilutions into test tubes containing 2.5 mL of ssMSA.
6. For each tube add 1 mL of bacterial culture of the host strain (in the case of heavily polluted water, which requires the addition of Nalidixic Acid Selective Supplement, use Escherichia coli WG5)
7. Distribute the entire contents of the tube onto the plates and ensure that the inoculum covers the entire surface.
8. Dry the plates in the incubator with the lid partially open and then close, invert and incubate at 36 ± 2 °C for 18 ± 2 hours. Do not stack more than six plates at a time.

10 - READING AND INTERPRETATION

Examinate the plates within 4 hours of the end of incubation and perform plaque counts using indirect light. To get an accurate result, consider only plates with a number of plaques between 30 and 300 units. Apply the MPN method.

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,

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
Escherichia coli ATCC 700078 (WG5)	37 ± 2 °C / 18-24H / A	good growth
Escherichia coli ATCC 13706	37 ± 2 °C / 18-24H / A	good growth

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

12 - LIMITATIONS OF THE METHOD

- ISO 10705-2 describes how to count plaques, however these can be difficult to see. In this regard, TTC (REF 42111801) can be added to the ssMSA to increase the contrast between the bacterial growth and the plaque-forming unit
- Mixing the ssMSA with the host strain and the water to be treated could cause the formation of lumps due to the different temperatures of the solutes. Make sure all compounds and reagents are at similar temperatures. If you need to use a bain-marie to dissolve the lumps, set a temperature of 45 ± 1°C and heat for no more than 10 minutes

13 - 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.
- The medium base and the supplement must be used in association according to the described directions.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before the use, consult the Material Safety Data Sheets.
- Apply Good Manufacturing Practice in the production process of prepared media.
- 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.
- Supplements are sterilized by membrane filtration.
- Be careful when opening the metal ring of the vials to avoid injury.
- All laboratory specimens should be considered infectious.
- Be careful when opening screw cap flasks to avoid injury from broken glass.
- When using a hot plate and/or double boiler, boil long enough to dissolve the entire medium.
- Wear heat protective gloves during the procedure. Do not place hot bottles in contact with ice or in cold water to speed cooling as this may cause the glass breaking.
- The time required for complete liquefaction of the medium can vary considerably and depends on the actual temperature of the heating device, its power, the size and volume of the bottle.
- Ready-to-use medium in flasks is sterilised by autoclaving.
- 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 tubes 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.

14 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

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).

Freeze-dried Nalidixic Acid Selective Supplement

Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

Calcium Chloride Solution

Upon receipt, store the product in the original package at +2°C /+8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Before use, examine the solution and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics)

Ready to use flask

Upon receipt, store in the original packaging at +2°C /+8°C protected from light. In these conditions the bottles are valid until the expiry date indicated on the label. Do not use beyond the expiry date. The bottles removed from the secondary packaging can be used until the expiry date. Opened bottles should be used immediately. Before use, check the closure and integrity of the screw cap. Discard bottles with signs of deterioration (e.g. microbial contamination, abnormal turbidity, atypical colour).

The user is responsible for the production and quality control processes of the prepared media and for validating their shelf life, based on the type and conditions of storage (temperature and packaging).

16 - REFERENCES

1. ISO 10705-2 , Water quality – Detection and Enumeration of bacteriophages, Part 2: enumeration of somatic coliphages
2. ISO 8199:2018 Water quality General requirements and guidance for microbiological examinations by culture.
3. ISO 5667-1:2023 Water quality Sampling Part 1: Guidance on the design of sampling programmes and sampling techniques
4. ISO 5667-3:2024 Water quality Sampling Part 3: Preservation and handling of water samples

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	Single use

REVISION HISTORY

Version	Description of changes	Date
Revision 0	First Issue	2024/05
Revision 1	Content updating	2024/10

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