MOTILITY MEDIUM

Dehydrated culture medium

1 - INTENDED USE

Semi-solid medium for motility test.

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

Pancreatic digest of casein	20.0 g
Enzymatic digest of meat	6.1 g
Agar	3.5 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

Since the early days of microbiology, the motility of bacteria has been used as a means of differentiation and classification.[1] In the early, motility was detected by microscopic observation, later in the late 1800s, with culture media containing agar, gelatin and various infusions. In 1935, Tittsler and Sandholzer[2] developed a semi-solid agar to determine motility by observing the spread of growth beyond the inoculation line.

Motility in bacteria develops through a variety of mechanisms, but the most common involve flagella which are mainly present in bacilli, but there are some flagellated cocci, so motility is a very important means of identification in the *Enterobacteriaceae* family.[1]

Motility Medium is prepared according to the formula described by ISO 11290 and is recommended in the procedure for confirmation of *Listeria* spp including *Listeria monocytogenes*.[3,4]

The medium contains casein and meat peptones which provide the essential growth factors for microbial growth. Small amount of agar helps to create a semisolid medium for motility test.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 29.6 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation, dispense into tubes in quantities of about 5 mL and sterilise by autoclaving at 121°C for 15 minutes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

6 - MATERIALS PROVIDED-PACKAGING

Product	Type	REF	Pack
Motility Medium	Dehydrated medium	4017142	500 g (16.9 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile inoculating needle, incubator and laboratory equipment as required, Erlenmeyer flasks, screwcap tubes, ancillary culture media and reagents.

8 – SPECIMENS

The specimens consist of colonies grown on plated media.

9 - TEST PROCEDURE

Using an inoculating needle, stab the medium with a pure culture obtained in Tryptic Soy Yeast Extract Agar (REF 402167).
Incubate at 25 °C for 48 h ± 2 h.

10 - READING AND INTERPRETATION

Examine for growth around the stab line.
A positive motility test is indicated by a diffuse growth outward away from stab line or turbidity of the medium; a negative motility test is indicated by growth confined to the stab line.
Listeria spp. are motile, giving a typical umbrella-like growth pattern. If growth is not sufficient, incubate for up to an additional five days and observe the stab again.

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
L. monocytogenes NCTC 7973	25°/ 48 H-A	good growth spreading beyond the inoculation line
L. innocua ATCC 33080	25°/ 48 H-A	good growth spreading beyond the inoculation line
E. coli ATCC 25922	25°/ 48 H-A	good growth spreading beyond the inoculation line
S. aureus ATCC 6538	25°/ 48 H-A	good growth, not motile

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 Motility Medium is tested for motility comparing the results with a previously approved Reference Batch.

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Pure colonies cultivated on Tryptic Soy Agar of 6 motile strains and 3 non-motile strains are inoculated by stabbing the medium in tubes. Motile strains: *L. monocytogenes* NCTC 7973, *L. innocua* ATCC 33090, *E. coli* ATCC 25922, *E. aerogenes* ATCC 13048, *P. mirabilis* ATCC 25933, S. Typhimurium ATCC 14028; Non-motile strains: *K. pneumoniae* ATCC 27736, *S. flexneri* ATCC 12022, *S. aureus* ATCC 25923. After incubation at 25°C for 24-48 hours, motility is observed and recorded. All strains exhibit performances characteristics according to the specifications.

13 – LIMITATIONS OF THE METHOD

- Some new *Listeria* species have been recently isolated. Most of them are not motile in the motility agar.[3,4]
- Motile bacteria but with damaged flagella can give false negative results.
- The presence of excess water in the tubes may lead to false positives.[1]
- The success of this test depends upon proper stab technique and the quality of the inoculating needle. It is recommended that a straight inoculating needle be used. If this is not available, consider using a disposable inoculating needle.[1]
- It is necessary to inoculate the medium taking care to remove the needle along the same stabbing line.
- Do not take inoculums from liquid or broth suspension.
- The isolated colonies on the plates should be identified with suitable tests.

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 production process of prepared 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 plates 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 the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging).

16 – REFERENCES

1. Shields P, Cathcart L. Motility Test Medium Protocol. 01 November 2011. American Society for Microbiology © 2016
2. Tittsler RP, Sandholzer LA. The use of semi-solid agar for the detection of bacterial motility. J Bacteriol 1936; 31:575–580.
3. ISO 11290-1:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 1: Detection method.
4. ISO 11290-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method.

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	2022/08

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

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