

TS-193000 rev 1 2023/03 page 1 / 2

# MONO CONFIRM TEST

## 1 - INTENDED USE

For the confirmation of *Listeria monocytogenes* and *Listeria* spp.

# 2 - COMPOSITION - KIT CONTENTS

12 strips with 8 microwells each for carry out 24 tests.

The following tests are performed into the microwells with the following dried substrates:

WELLS	TESTS	SUBSTRATES	
A or E	Acid production from arabitol (ARA)	D-arabitol	
B or F	Acid production from α-methyl- D-glucoside (MEGLU)	α -methyl-D-glucoside	
C or G	Beta-glucosidase (X-GLUPY)	5-bromo-4-chloro-3-indolyl-beta-D-glucopyranoside	
D or H	D-aminopeptidase (AMP)	aminoacil β- naphtylamide	

1 frame with the lid

• p-dimethylaminobenzaldehyde 5% solution in HCl 10%, 4 mL

24 plastic pipettes

。Standard Mac Farland n. 2

• 1 Product Information and result reporting paper

#### **3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE**

Mono Confirm Test is a micro method based on four biochemical reactions for confirmation of *Listeria* spp. and differentiation of *Listeria* monocytogenes from other non-*L. monocytogenes* species. Substrates are dried in plastic microwells; the kit provides 12 strips with 8 microwells; 2 strains can be tested with each strip. *Listeria* spp. is differentiated from other genera by  $\beta$ -glucosidase test and acid production from arabitol and  $\alpha$ -methyl D-glucoside. *L. monocytogenes* is identified by detection of a new specific enzyme D-aminopeptidase, which is present in all non-pathogenic *Listeria* species and absent in *L. monocytogenes* strains.

## **4 - PHYSICAL CHARACTERISTICS**

Appearance of p-dimethylaminobenzaldehyde 5% solution

limpid, very pale-yellow solution

# **5 - MATERIALS PROVIDED - PACKAGING**

Product	Туре	REF	Pack
Mono Confirm Test	Kit	193000	24 tests

# 6 - MATERIALS REQUIRED BUT NOT PROVIDED

Tubes, sterile saline solution, sterile swabs, incubator, ancillary culture media and reagents.

#### 7 - SPECIMENS

The specimens consist of pure colonies grown on plated media.

#### 8 - TEST PROCEDURE

- 1. For confirmation of presumptive *L. monocytogenes* or Listeria spp., take at least one colony from selective medium (up to a maximum of five colonies from each plate of each selective medium).
- 2. Streak the selected colonies onto the surface of pre-dried plates of a non-selective agar, for example TSYEA (Tryptic Soy Yeast Extract Agar REF 402166) for obtaining well isolated colonies.
- 3. If required check for Gram staining, motility, catalase and oxidase. *Listeria* strains are short Gram-positive rods, motile at 25°C but not at 37°C, catalase positive, oxidase negative.
- 4. With the colonies cultivated on TSYEA, prepare 1 mL of bacterial suspension in sterile saline solution with a turbidity equal to Mc Farland n° 2 (between 6 and 8 colonies.)
- 5. Transfer the needed strips onto the frame
- 6. Distribute 3 drops of bacterial suspension into the wells A-B-C-D or E-F-G-H by means of the supplied plastic pipettes.
- 7. Cover the inoculated strips with the lid and incubate at 37°C for 18 24 hours
- 8. Read the reactions which do not need the additional reagent in the wells A-B-C (or E-F-G): ARA, MEGLU, X-GLUPY
- 9. Add one drop of dimethylaminobenzaldehyde reagents to the well D (or H) and read the reaction AMP
- 10. Record the positive and negative results and identify the strains according to the below schemes
- 11. Wash with a disinfectant the frame and the lid after the use.

# 9 - READING AND INTERPRETATION

F	REA	DI	NG	

TEST	POSITIVE REACTION	NEGATIVE REACTION
Arabitol (ARA)	straw colour	blue or blue-green colour
Methyl-glucoside (MEGLU)	straw colour	blue or blue-green colour
Beta-glucosidase(X-GLUPY)	blue - clear blue colour	colourless
D-aminopeptidase (AMP)	yellow colour	Colourless
	(after dimethylaminobenzaldehyde addition)	(after dimethylaminobenzaldehyde addition)





# **RESULTS INTERPRETATION**

ARA	MEGLU	X-GLUPY	D-AMP	IDENTIFICATION
-	-	-	*	Not-Listeria sp.
-	+	+	*	Not- <i>Listeria</i> sp.
-	-	+	*	Not- <i>Listeria</i> sp.
-	+	-	*	Not- <i>Listeria</i> sp.
+	-	-	*	Not- <i>Listeria</i> sp.
+	-	+	*	Not- <i>Listeria</i> sp.
+	+	-	*	Not- <i>Listeria</i> sp.
+	+	+	+	Listeria sp. not-monocytogenes
+	+	+	-	Listeria monocytogenes

\* When even one of the tests ARA, MEGLU, X - GLUPY is negative, do not read the AMP reaction

#### **10 - 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 the test strains useful for the quality control.

Positive control:L. monocytogenes ATCC 13932 or ATCC 19111Negative control:L. innocua ATCC 33090

ATCC is a trademark of American Type Culture Collection

## **11 - PERFORMANCES CHARACTERISTICS**

Prior to release for sale a representative sample of all lots of Mono Confirm Test is tested with positive and negative strains.

Pure cultures of the strains shown below are inoculated in the wells and colour development is recorded after 18-24 hours of incubation *L.monocytogenes* strains: ATCC 19111, ATCC 13932, ATCC 7644, CB 001, CB002,

Listeria non-monocytogenes strains: L.innocua ATCC 33090, L.gray CB007, L.ivanovii ATCC 19119, L.murray CB 008, L.seeligeri ATCC 35967, L.welshimeri CB 006.

Non-Listeria strains: S.aureus ATCC 25923, E.faecalis ATCC 19433, P.aeruginosa ATCC 27853

All tested bacterial strains develop coloration in the wells in accordance with specifications.

## **12 - LIMITATIONS OF THE METHOD**

 According to ISO 11290, isolates which are considered to be L. monocytogenes may be sent for further characterization to a recognized national or regional Listeria Reference Laboratory.<sup>1</sup>

# **13 - PRECAUTIONS AND WARNINGS**

- This product is for laboratory use and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is classified as dangerous according to the current European legislation. Consult the Safety Data Sheet before the use.
- Apply good laboratory practice guidelines when performing the test.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as the reagents, the culture media or the microbial strains.
- Sterilize all biohazard waste before disposal. Dispose the used and unused strips and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet 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

Upon receipt, store the product in the original pack with the cap tightly closed, at +2/+8°C away from direct light. If properly stored, the product may be used up to the expiration date. Do not use the product beyond this date.

# 15 - REFERENCES

1. 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.

REF or REF	LOT Batch code	Manufacturer	This side up	Fragile, handle with care
Catalogue number				
Temperature	Content sufficient for <n> tests</n>	Consult Instructions for Use	Use by	Keep away from direct light

REVISION HISTORY			
Version	Description of changes	Date	Date
Revision 3	Updated layout and content	2023/03	2023/03
Note: minor typographical, grammatical, and formatting			

