Listeria Monocytogenes

For in *Vitro* diagnostic use only

Immunochromatographic rapid test for the qualitative detection of *Listeria Monocytogenes* in faecal and food samples

I. INTRODUCTION AND INTENDED USE

Listeria monocytogenes is a small, gram-positive bacillus that can grow in anaerobic or aerobic conditions. It is found widely in the environment in soil, decaying vegetation and water and may be part of the fecal flora of many mammals, including healthy human adults. Initial symptoms of infection include nonspecific flu-like symptoms, nausea, vomiting, cramps, diarrhea and fever. There are few clinical features that are unique to listeriosis. Therefore, clinicians must consider a variety of potential causes for infection, including viral infections (influenza) and other bacterial infections that may cause sepsis or meningitis. Symptoms can develop at any time from 2 to 70 days after eating contaminated food. Except for vertical mother–fetus transmission, most cases of listeriosis begin with ingestion of the organism from a food source.

Most healthy adults and children who consume contaminated food experience only mild to moderate symptoms. People with poor immune function are at much higher risk of severe, life-threatening forms of listeriosis.

Listeria Device provides a rapid detection of *Listeria monocytogenes* from enrichment food samples, and directly from faecal samples.

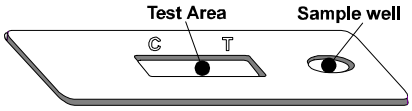
The Listeria Monocytogenes Kit Mascia Brunelli is a one step coloured chromatographic immunoassay for the qualitative detection of *Listeria monocytogenes* in faecal samples and in contaminated food samples, after enrichment.

II. PRINCIPLE OF THE TEST

The Listeria Monocytogenes is a qualitative immunoassay for the determination of *Listeria monocytogenes (L. monocytogenes)* in faecal and food samples. The membrane is pre-coated with mouse monoclonal antibodies, on the test band region, to recognize this antigen.

During testing, the sample is allowed to react with the coloured particles coated anti-*L. monocytogenes* antibodies which were pre-dried on the strip. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the coloured particles conjugate migrate. In the case of a positive result the specific antibodies present on the membrane will capture the coloured particles (conjugate). The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, a green coloured band always appears. A green coloured band always appears in the control line and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

III. REAGENTS AND MATERIALS



Each kit contains:

1. Listeria Monocytogenes (10 card)
2. 1 bottle with Extraction buffer (8 mL)
3. Instruction for use (1)

Required materials (not supplied)

Testing tubes, specimen collection container, disposable gloves and container, plastic pipette and timer.

Listeria Fraser broth half concentration (Mascia Brunelli-Biolife Ref. 5115943), Bigmixer1 (Mascia Brunelli-Biolife Ref.7221230), Medium Aloa (Mascia Brunelli-Biolife Ref. 541605), Brain Heart Infusion Broth (Mascia Brunelli-Biolife Ref. 551230), Tryptic Soy broth (Mascia Brunelli-Biolife Ref. 552165), Listeria Fraser Broth (Mascia Brunelli-Biolife Ref. 551594), incubators +30ºC and +37ºC. Purified water.

IV. SPECIAL PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after expiration date.
- The test should remain in the sealed pouch until use.
- Do not use the test if pouch is damaged.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, do not eat, drink or smoke in the area.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The test should be discarded in a proper biohazard container after testing.
- The test must be carried out within 2 hours of opening the sealed bag.

V. STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30ºC/36-86ºF). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

VI. SPECIMENS COLLECTION FOR FAECAL SAMPLES

Collect sufficient quantity of faeces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-4ºC/36-40ºF) for 1-2 days prior to testing. For longer storage the specimen must be kept frozen at –20ºC/4ºF. Freezing and thawing cycles are not recommended. The sample will be totally thawed, brought to room temperature and mix as thoroughly as possible before testing.

VII. PROCEDURE FOR FAECAL SAMPLES

Procedure of the samples

Use a separate swab or stick, pipette, vials for each sample. Dispense 0.7mL (or 14 drops) of extraction buffer into a testing tube. Collect the stool sample with the swab or stick by dipping in two different places of the same stool specimen. Verify to transfer a small portion (150 mg) of stool in testing tube with extraction buffer. Shake the extraction tube in order to get an homogeneous solution. For liquid or semi-solid stools using a separate pipette, draw stool of the sample itself. Dispense 150 µl of each stool into a testing tube with 1.0ml (or 20 drops) of extraction tube. Mix carefully, then vortex 15 seconds.

Procedure of the test

Allow the tests, stool samples and buffer to reach to room temperature prior to testing.

1. Remove the *Listeria Monocytogens* from its sealed pouch and use it as soon as possible.
2. Use a clean material for each sample.
3. Take a quantity of liquid from the topside with a dropper and dispense exactly 4 drops into the specimen well (S). Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.

VIII. SPECIMENS COLLECTION FOR FOOD SAMPLES

Food samples should be collected in clean containers and the assay should be done right after collection. The samples can be stored in the refrigerator (2-4 ºC) for 1-2 days prior to testing. For longer storage, the specimen must be kept frozen at –20ºC. In this case, the sample will be totally thawed, and brought to room temperature before testing. Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended. Homogenise sample as thoroughly as possible prior to preparation.

Sample enrichment:

- Mix 25 g solid sample or 25 mL liquid sample with 225 mL of half-concentration FRASER broth and homogenise with a Bagmixer 1 for 2 minutes if necessary.

- Incubate for 21-24 h at 28-30°C.
- Transfer 0.1mL of enrichment broth to the surface of ALOA plates.
- Incubate for 24±2 hours at +37°C. If no typical colonies are present after 24 h of incubation or if no growth occurs, re-incubate the plates for further 24±2 hours.

Confirmation culture

- Pick up 1-3 suspect colonies from isolation media (ALOA).
- Resuspend in 0,25 mL of Brain Heart Infusion broth or Tryptic Soy broth or Listeria Fraser broth and mix.
- Incubate for 1 h at +37ºC.
- Allow to cool to room temperature.

IX. PROCEDURE FOR FOOD SAMPLES

Allow the devices, samples and controls to reach to room temperature prior to testing.

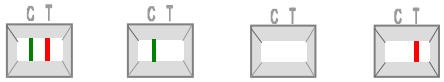
1. Place 1.0-2.0 mL (approx. 30-40 drops) of enrichment sample or confirmatory sample in a testing tube.
2. Remove the Listeria Monocytogenes from its sealed pouch and use it as soon as possible.
3. Use a separate pipette and device for each sample or control. Dispense 3-4 drops or 100uL from the enrichment sample or confirmatory sample into the specimen well (S). Start the timer.
4. Read the result at 10 minutes after dispensing the sample. (Coloured bands appear)

X. INTERPRETING THE RESULTS

NEGATIVE: Only one green control band appears across the central window in the site marked with the letter C (control line).

POSITIVE: In addition to the green control band across the central window in the site marked with the letter C (control line), a red band (test line) also appears in the site marked with the letter T (result region).

INVALID: A total absence of the control coloured band. Review the procedure and repeat the tests using a new test.



Positive Negative Invalid

XI. INTERNAL QUALITY CONTROL

Internal procedural controls are included in the test. a green line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

XII. PERFORMANCE

Detection limit

The detection limit test is *L. monocytogenes* 6.25x10^{4}bacteria/mL.

Sensitivity and specificity

It was studied some samples using *Listeria Monocytogenes*. For all samples, the result was confirmed by Singlepath® L'mono (Merck). The results were >99% of sensitivity and >96% of specificity.

The use of a mouse monoclonal antibody in *Listeria Monocytogenes* assures high degree of specificity for the detection of these bacteria. The antibodies used to elaborate this test recognise *Listeria* epitopes found in stool of patients, as well as in preparations from the bacteria cultures in vitro.

This preliminary values has to be taken with precaution until more evaluation data will be available.

Cross-reactivity

It was performed an evaluation to determine the cross reactivity of *Listeria Monocytogenes*. There is not cross reactivity with common intestinal pathogens, other organisms and substances occasionally present in faeces: *H. pylori, Escherichia coli O157:H7, Astrovirus, Rotavirus, Adenovirus, Campylobacter, Salmonella.* There is not cross reactivity with common food pathogens, other organisms and substances occasionally present in foods: *Escherichia coli O157, Campylobacter and Salmonella.*

XIII. LIMITS OF THE TEST

- The test must be carried out within 2 hours of opening the sealed bag.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- Some stool samples can decrease the intensity of the control green line.
- Freezing and thawing cycles for the sample are not recommended, it could cause wrong results.
- A negative result is not meaningful because it is possible the *Listeria m.* content in the stool sample to be too small. A *Listeria m.* determination should be carried out on a sample from an enrichment culture.
- This test provides a presumptive diagnosis of Listeriosis or absence or presence of *Listeria monocytogenes* in food sample. A confirmed infection diagnosis or positive result should only be made by a physician after all clinical and laboratory findings have been evaluated must be based in the correlation of the results with further clinical observations.

XIV. REFERENCES

1. BOTTELDOORN N, et al. "Microbiological and molecular investigation of an increase of human listeriosis in Belgium, 2006-2007". Euro Surveill. 2010;15(6):pii=19482.
2. .BORTOLUSSI, R. "Listeriosis: a primer". CMAJ, October , 2008 Vol 179(8), pp 795-797

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (10 tests) **Ref. VQ84050**

Listeria Monocytogenes	10 Device (Card)
Extraction buffer	1 bottle (8 mL)
Instruction for use	1 item

EDMA Code 15 01 13 01 00 CE IVD