

Singlepath® L'mono

For the rapid detection and confirmation of *Listeria monocytogenes* in foods

Introduction

Listeria are gram-positive, nonspore-forming, rod-shaped bacteria. Of the six known species of the *Listeria* genus, *Listeria monocytogenes* deserves particular mention as a human and animal pathogen, while *L. ivanovii* is pathogenic only in animals and *L. innocua*, *L. seeligeri*, *L. grayi*, and *L. welshimeri* are considered harmless environmental bacteria.

Listeriosis, the disease caused by *L. monocytogenes*, manifests itself not only as sepsis, but also and primarily as meningitis or even as encephalitis. Since *L. monocytogenes* is capable of crossing the placenta barrier, an infection of the pregnant mother with listeria constitutes a special risk for the fetus or result in the infection of the newborn child. *L. monocytogenes* is also responsible for severe infections in immunocompromised persons. As a result of the ubiquitous distribution of *Listeria* and their capability to grow at refrigerator temperatures (+2°C to +8°C), foods constitute one of the main sources of infection.

Due to this fact the detection of *Listeria monocytogenes* in foods and environmental samples is absolutely necessary. The drastic increase in the incidence of food infection caused by *Listeria* demands reliable and rapid methods of detection. Apart from traditional culture methods, immunological techniques are becoming more and more popular with users due to their better specificity and sensitivity.

Singlepath® L'mono is an immunological screening and an extremely fast confirmation test for *Listeria monocytogenes* based on the immune flow principle and is designed in such a way that time-consuming and personnel intensive working steps for the application and interpretation of the tests are avoided.

Mode of Action

Singlepath® L'mono is an immunochromatographic rapid test based on gold-labelled antibodies. The test device has a circular sample port, and an oval shaped test (T) and control (C) window.

1. The sample is applied to the chromatography paper via the circular sample port
2. The sample is absorbed through the pad to the reaction zone containing colloidal, gold-labelled antibodies specific to *Listeria monocytogenes*.
3. Any *Listeria monocytogenes*-antigen present complexes with the gold-labelled antibody and migrates through the port until it encounters a binding zone in the test (T) area.
4. The binding zone (T) contains another anti-*Listeria monocytogenes* Antibody, which immobilises any *Listeria monocytogenes*-antibody complex present. Due to the gold-labelling, a distinct red line is then formed.
5. The rest of the sample continues to migrate to a second binding reagent zone within the control (C) zone, and also forms a second distinct red line (positive control). Regardless of whether any *Listeria monocytogenes* is present or not, this distinct red line is always formed in the control (C) zone, thus ensuring the test is working correctly.

Sample material / sample enrichment

Screening assay

- Mix 25 g solid sample or 25 ml liquid sample with 225 ml of half-concentrated FRASER broth or bLEB or L-PALCAM broth or UVM-I broth and homogenise with a Stomacher if necessary.
- Incubate for 21-24 h at +28 - 30°C or at 35-37°C.
- Transfer 0,1 ml in 10 ml buffered LEB or Fraser broth or L-PALCAM broth or UVM-II broth.
- Incubate for 21-24 h at +28 - 30°C or at 35-37°C.
- Allow to cool to room temperature

Confirmation assay

A wide range of foods may be tested for *Listeria spp.* or *Listeria monocytogenes* using *Listeria* selective agars (e.g. PALCAM, Oxford, Chromoplate *Listeria*, Chromocult *Listeria*, etc.). Single-path L'mono can be used for confirmation of suspect *Listeria monocytogenes* colonies on these agars.

- Pick up 1-3 suspect colonies
- Re-suspend in 250 µl Brain-Heart (BHI) broth or CASO or L-PALCAM or Full Fraser broth and mix.
- Incubate for 1 h at 37°C
- Allow to cool to room temperature

Experimental Procedure and Evaluation

Sample preparation

Prior to use, allow the enriched sample and test device to reach room temperature (15-25°C)

Procedure

1. Remove the foil pouches from the required number of Singlepath® L'mono devices. Place the test device(s) on a flat surface and label with appropriate sample identification. (Note: Perform the tests within a period of 2 hours after opening!)
2. Using a micro pipette and disposable pipette tip, draw up 150 µl from the enrichment broth.
3. Dispense 150 µl of the sample into the circular sample port on the test device. Alternatively using a disposable transfer pipette, squeeze the pipette bulb, insert the stem into the sample and release pressure on bulb. This will draw sample up into the pipette. Dispense 5 free falling drops (about 150 µl) into the circular sample port on the test device.
4. Observe the test result within 30 minutes after applying the sample to the device.

Interpretation of results

The test can be regarded as working correctly if a distinct red line appears in the control zone (C) within 30 minutes.

A sample can be considered POSITIVE if at or prior to 30 minutes, red lines appear on both test (T) and control (C) zones.

A sample can be considered NEGATIVE if no red line appears in the test (T) zone but does appear distinctly in the control (C) zone 30 minutes after application of sample to the device.

Any positive result obtained with the screening assay should be confirmed by a validated culture method.

Technical specifications

Detection limit

Depending on serotype, approx. 5×10^6 bacteria/ml or 1 colony on agar plate can be regarded as being the lower detection limit. Negative results may occur if the amount of antigen extracted is below the minimum sensitivity of the tests.

Interferences

Results obtained to date on numerous food samples indicate that there is no interference of Singlepath® L'mono with food ingredients.

The test has been developed based on using bLEB, UVM, L-PALCAM and Fraser selective enrichment broth from MERCK. Interference from other types of selective enrichment broths and other brands cannot be excluded. In particular use of broth of red-brown colour could potentially mask weak signals due to background coloration of the test zone.

Singlepath® L'mono has demonstrated not to detect *Listeria monocytogenes* in screening assays at lower concentration in raw beef sample/ground beef and other foods with high background flora.

Trouble-shooting

Problem	Measures
No line appears in either zone after within 25 minutes test period	Re-run sample
Delay in sample reaching	Touch sample pad with pipette tip Nitrocellulose membrane

Precautions

Users of Singlepath® L'mono must be familiar with the appropriate aseptic techniques for the isolation and identification of *Listeria monocytogenes*. Care must be taken when handling samples, enrichments and devices.

Disposal

Decontaminate Singlepath® devices, enrichments, tubes, and pipettes by autoclave, bleach etc in accordance with local, state, and federal regulations.

Technical assistance

For technical assistance, please contact your local Merck representative or Merck KGaA, 64271 Darmstadt, Germany.

Tel.: +49-6151-720, Fax : +49-6151-722000,

Email: service@merck.de

Ordering Information

Product	Merck Cat. No	Pack size
Singlepath® L'mono	1.04148.0001	25 tests
Singlepath® Listeria	1.04142.0001	25 tests
Brian Heart Broth (BHI)	1.10493.0500	500 g
Listeria enrichment broth, buffered (base)	1.09628.0500	500 g
Listeria selective enrichment supplement	1.11781.0001	16 vials
Fraser Listeria selective enrichment broth (base)	1.10398.0500	500 g
Fraser Listeria supplement	1.10399.0001	16 vials
L-PALCAM Listeria selective enrichment broth (base) acc. to van Netten et al.	1.10823.0500	500 g
PALCAM Listeria selective supplement	1.12122.0001	16 vials
UVM-Listeria selective enrichment broth modified	1.10824.0500	500 g
UVM-II supplement	1.04039.0001	16 vials
Chromoplate® Listeria Selective Agar acc. to Agosti and Ottaviani	1.00420.0020	20 plates
Chromocult® Listeria	1.00427.0500	500 g
Oxford Listeria agar	1.07004.0500	500 g
Oxford Listeria selective supplement	1.07006.0001	13 vials
PALCAM Listeria agar acc. to van Netten et al.	1.11755.0500	500 g
PALCAM Listeria selective supplement	1.12122.0001	16 vials

Singlepath® L'mono

Additionally required materials and instrumentation

1. Enrichment media, e.g. 1. 09628 Listeria enrichment broth, buffered (base) acc. to FDA/BAM 1995 (bLEB), 1.11781 Listeria selective enrichment supplement acc. to FDA/BAM 1995, 1.10398 FRASER Listeria selective enrichment broth (base), 1.10399 FRASER Listeria Supplement, 1.10823 L-PALCAM Listeria selective enrichment broth (base), 1.12122 PALCAM-Listeria-Selective-Supplement acc. to van Netten et al., 1.10824 UVM Listeria selective enrichment broth modified, 1.04039 UVM-II Supplement
2. Stomacher / Stomacher bags
3. Incubators +28°C - 37°C
4. Distilled or deionized water
5. Autoclave
6. Disposable plastic transfer pipettes and/or appropriate micro-pipettes and disposable tips
7. Disposable inoculation loops



Singlepath® L'mono: Test result negative for Listeria monocytogenes



Singlepath® L'mono: Test result positive for Listeria monocytogenes