INTENDED USE

The Oxoid Listeria Rapid Test is designed for the detection of *Listeria* species in foods and environmental samples within 43 hours. The test protocol allows for the availability of the result two working days after the sample is received in the testing laboratory. The procedure uses two carefully selected enrichment steps for the maximum recovery and growth of *Listeria* followed by an immunoassay in the Clearview™ format. This simple system gives a clear visual result 20 minutes after the addition of the heated and cooled sample to the test device with no further manipulations being required.

SUMMARY

*Listeria* is a genus of gram-positive, non-sporing bacilli with a DNA G+C content of 36-38%. They have up to 6 peritrichous flagella and are motile when grown at 30 degrees C. or below. They are aerobic and facultatively anaerobic, catalase-positive and oxidase-negative. The genus comprises six species, *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri* and *L. grayi* subsp. *grayi* and *L. grayi* subsp. *murrayi*. All *Listeria*, except *L. grayi*, variously share 4 flagella antigens A, B, C, and D, of which flagella antigen B is common.\(^{(1)}\)

Pathogenic and non-pathogenic *Listeria* are ubiquitous in nature and can be isolated from soil, vegetables and natural waters as well as for healthy animals and man. They are able to grow over a temperature range of 1-45 degrees C. Consequently, *L. monocytogenes* is a food poisoning risk to susceptible individuals if present in food that are subsequently stored at these temperatures for sufficient time for the organism to grow to infectious levels before ingestion. Clinical symptoms include flu-like illness, spontaneous abortion, still birth, meningitis, pneumonitis, septicemia and endocarditis. *Listeria monocytogenes* infections mainly occur in neonates, pregnant women, the elderly and immunocompromised individuals.

TEST PRINCIPLE

**Enrichment Broth System**
Culture of the test sample is in two sequential enrichments, taking 42 hours. Any *Listeria* organisms present in the food or environmental sample are selectively enriched using growth conditions which are optimal for flagella expression.

**Antigen Extraction**
The second enrichment media is heated at 80 degrees C. for 20 minutes to extract the flagella antigen.
Clearview™ Listeria Device
The Clearview™ Listeria device contains specific monoclonal antibodies to the B flagella antigen that is common to the *Listeria* species indicated earlier.\(^2\)

The extracted antigen is added onto a pad in the Sample Window. This contains blue latex labelled with antibody. The extract rehydrates the complex and the specific antigen reacts, if present, with the antibody.

The complex moves through the pad by capillary action to a test strip containing an immobilized line of antibody midway along the Result Window.

A further reaction between antigen/latex complex and the fixed antibody results in a blue line in the Result Window.

If no flagella antigen is present, the Result Window will remain clear.

The Clearview™ Listeria device also provides an integral control feature. The appearance of a blue line in the Control Window shows the test has been carried out correctly.

**COMPONENTS OF THE KIT**

- Oxoid SR0166 Half Fraser Supplement: 50 vials
- Half Fraser Supplement is used in conjunction with Fraser Broth (CM0895), it is modified by the addition of only half the level of selective agents normally found in Fraser Supplement. Each vial is sufficient for 225ml of broth.
- Listeria Test Units: 50
- Positive Clearview™ Control: 3 vials
- Non-viable *Listeria monocytogenes* suspension.

**STORAGE AND SHELF LIFE**

This kit must be stored at 2-8 degrees C. Under these conditions, the components of the kit will retain activity until the date shown on the box. Do not freeze.

This product has the following shelf life from the date of manufacture:

<table>
<thead>
<tr>
<th>Days</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>540</td>
<td>FT401M Listeria Rapid Test</td>
</tr>
</tbody>
</table>

**PRECAUTIONS**

*Pregnant women should not be in contact with this organism in the laboratory.*

For *in vitro* use only.
Standard guidelines for the safe handling and the disposal of infectious microorganisms should be observed throughout all procedures.

Do not mix kit components from different lots.

Do not use after the expiry date stated on the kit.

PROCEDURE

A) Media Preparation

Primary Enrichment - Half Fraser Broth
FB CM0895 should be prepared in accordance with the manufacturer's instructions and dispensed into appropriate volumes prior to sterilization. Reconstitute the Half Fraser Supplement (SR0166) by adding 4ml of the 1:1 ethanol/water mixture to a vial. This is sufficient for 225ml of broth. Add an appropriate amount of supplement to the dispensed volumes of FB immediately prior to use.

Secondary Enrichment Broth (BLEB)
BLEB - CM0897 should be prepared in accordance with the manufacturer's instructions and dispensed into 10ml volumes prior to sterilization. Reconstitute the BLEB Supplement (SR0141) in accordance with the manufacturer's instructions. Add 40ml of the reconstituted BLEB Supplement to 10ml of BLEB immediately prior to use.

B) Sample Culture System
Food samples are prepared by making a homogenate of the food in the Half Fraser Broth, as described below (see Table 1).

Environmental swabs should be quenched in 10ml of an appropriate neutralizing agent and the whole added to 90ml of Half Fraser Broth (see Table 1).

1. Dilute the test sample in 1-10 weight/volume of the prepared Half Fraser Broth and stomach for the appropriate time according to the sample type.

2. Incubate the mixture at 30 degrees C. for a minimum of 21 but not exceeding 24 hours.

3. Transfer 0.1ml of the Half Fraser Broth into 10ml of prepared BLEB.

4. Incubate the BLEB at 30 degrees C. for a further 21 but not exceeding 24 hours.

<table>
<thead>
<tr>
<th>Foods</th>
<th>Swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Swab + 10ml neutralizing agent +</td>
</tr>
<tr>
<td>25g + 225ml 1/2FB*</td>
<td>90ml 1/2FB</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>30 degrees C.</td>
<td>21-24 hrs</td>
</tr>
<tr>
<td>0.1ml</td>
<td>10ml BLEB</td>
</tr>
<tr>
<td>30 degrees C.</td>
<td>21-24 hrs</td>
</tr>
<tr>
<td>2ml</td>
<td>glass tube (bijou)</td>
</tr>
<tr>
<td>80 degrees C.</td>
<td>20 mins</td>
</tr>
<tr>
<td>Cool</td>
<td>135ul</td>
</tr>
<tr>
<td>Listeria Device (20 mins at RT)</td>
<td></td>
</tr>
</tbody>
</table>

* Half FB Supplement - 1 vial per 225ml FB Supplement reconstitute in 4ml 1:1 ethanol water.

**C) Preparation of the BLEB sample for Clearview™ testing**
1. Carefully remove the incubated BLEB from the incubator without disturbing any food debris present. Transfer 2ml of the upper region of the broth to a small glass tube.

2. Place the glass tube in the waterbath at 80 degrees C. for 20 minutes.

3. Allow the extract to cool to room temperature.

**D) Clearview™ Listeria Device Test Protocol**
1. Ensure that the device is at room temperature before beginning the assay.

2. Remove a Clearview™ test unit from the foil wrapper and place on a level surface. Label with the identity of the test sample.

3. Pipette 135ul of the BLEB extract onto the Sample Window. If any precipitate or viscous material is present after heating, avoid pipetting this into the device.

4. After 20 minutes, examine for the presence of a blue line of any intensity in the Result Window.

**INTERPRETATION OF RESULTS**
A blue line in the Result Window indicates the presence of *Listeria* flagella antigen in the BLEB. This is a positive result. If identification at species level is required, the *Listeria* organisms may be subcultured from the BLEB Broth onto a selective agar, e.g. Oxford Agar. Colonies resembling *Listeria* should be purified on non-selective media and appropriate confirmation and specification tests carried out.

A blue line in the Control Window indicates that the device has worked correctly.

Differences in the intensity of the blue lines in the Result and Control Windows may occur, but this does not affect the interpretation of the result. A very strong blue line in the Result Window with no line in the Control Window indicates an excess of flagella antigen. This is an uncommon event (typically less than 0.1%). If reassurance that the test has worked properly is required, the sample may be retested with a dilution step. The heated extract should be diluted 1 in 10 in fresh BLEB and retested on another device.

If no line appears in the Result or Control Window within 20 minutes, a further Clearview™ device should be set up using the same culture extract, providing this is not more than 1 hour old.

**LIMITATIONS**

The Clearview™ Listeria device is only recommended for use with the Oxoid enrichment broths and supplements that are described in the above protocol.(3)

Negative results may occur if the amount of antigen extracted is below the minimum sensitivity of the test, or if incubation temperatures above 30 degrees C. are used.

False-negative results may be obtained when testing concentrated yeast materials by this method. This can be improved by extending the incubation times to 24-26 hours for both primary and secondary enrichment broths. The Clearview™ Listeria device has demonstrated not to detect *Listeria* at a lower concentration in raw beef samples. *Listeria grayi* is not detected.

Do not use test units that have become wet, or removed from their foil wrapper on a previous day.

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Oxoid Fraser Broth (FB), CM0895
- Oxoid Buffered Listeria Enrichment Broth (B.L.E.B.), CM0897
- B.L.E.B. Selective Enrichment Supplement, SR0141
- Incubator at 30 +/- 2 degrees C.
- Waterbath at 80 +/- 2 degrees C.
- Glass Test Tubes of 5-8ml capacity
- Sterile Water/Ethanol Mixture 1:1 (v/v)
QUALITY CONTROL

A positive (non-viable) flagella freeze-dried antigen control is included in the kit. Reconstitute a vial with 2ml of sterile distilled water. The reconstituted positive control should be stored at 2-9 degrees C. Under these conditions, it will retain its activity for 6 months. Add 135ul of positive control reagent onto the Sample Window of a separate device.

If a negative control is required, add 135ul of uninoculated BLEB onto a separate device. Do not use any other reagents as a negative control.

PERFORMANCE CHARACTERISTICS

The Oxoid Listeria Rapid Test has been evaluated in a wide variety of foods, comparing the Clearview™ device with isolation on Oxoid Oxford agar from the secondary enrichment broth. More than 1000 tests have given >99% correlation.\(^{(4)}\)

The antibodies in the Clearview™ device has been tested for cross-reactivity against a panel of organisms listed below. The organisms were at a minimum level of \(1 \times 10^8/\text{ml}\). No cross-reactivity was observed with any of the organisms.

<table>
<thead>
<tr>
<th>Acinetobacter anitratus</th>
<th>Enterobacter aerogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerococcus viridans</td>
<td>Enterobacter cloacae</td>
</tr>
<tr>
<td>Arizona spp.</td>
<td>Enterococcus faecalis</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Bacillus circulans</td>
<td>Hafnia alvei</td>
</tr>
<tr>
<td>Bacillus licheniformis</td>
<td>Klebsiella pneumoniae</td>
</tr>
<tr>
<td>Bacillus macerans</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>Proteus stuartii</td>
</tr>
<tr>
<td>Citrobacter diversus</td>
<td>Salmonella spp.</td>
</tr>
<tr>
<td>Citrobacter koseri</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Edwardsiella tarda</td>
<td>Streptococcus faecalis</td>
</tr>
</tbody>
</table>
Non-*Listeria* organisms - 7 esculin positive isolates from Oxford Agar.

REFERENCES


This document is provided for general product information only. It does not replace the manufacturer's product insert. Always refer to the actual product insert for procedural use and for most recent product information.

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