**KB012A HiListeria™ Identification Kit**

**Introduction**

*Listeria* species are ubiquitous organisms and are the most frequent contaminants of various kinds of food products. Human Listerialiosis occurs in sporadic and epidemic forms and has 20% to 30% fatality rate. The pathogenic strain of humans, *L. monocytogenes* primarily causes Meningitis, Encephalitis, Septicemia, and in pregnant women it may cause abortion, still birth or premature birth. KB012A can be used for screening food samples and other relevant clinical samples. It can also be used for validating known laboratory strains. The complete list of organisms that can be identified with this kit is given in the identification index provided with the kit.

**Principle**

Each KB012A is a standardized, colorimetric test system based on motility, carbohydrate utilization and other biochemical tests specific for the identification of *Listeria* species. The tests are based on the principle of pH change and substrate utilization. *Listeria* species on incubation exhibit metabolic changes which are indicated by a colour change in the media that can be either interpreted visually or after addition of reagent wherever required.

**Kit Contents**

1. Each kit contains sufficient material to perform 10 tests.
2. 10 kits of KB012A
3. Technical product insert
4. Result Interpretation Chart and Result Entry Datasheet
5. Identification Index

**Instructions for use**

**Preparation of inoculum**

KB012A cannot be used directly on clinical or food samples. The organism to be identified has to be first isolated and purified. Isolate the organism to be identified on either PALCAM Agar (M1064) or Tryptose Agar (M538) with or without blood. Pick up a single isolated colony and inoculate in 5 ml Brain Heart Infusion Broth (M210) and incubate at 35-37°C for 6 to 8 hours until inoculum turbidity is ≥1.0 OD at 620nm.

**Method of Inoculation**

- Open the kit aseptically. Peel off the sealing foil.
- Inoculate each well with 50µl of the above inoculum by surface inoculation method.
- Alternately, the kit can also be inoculated by stabbing each individual well with a loopful of inoculum.

**Incubation**

- Temp. of Incubation: 35-37°C. Duration of Incubation: 24-48 hrs.

**Identification Index of various Listeria species**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Catalase</th>
<th>Nitrate Reduction</th>
<th>Esculin Hydrolysis</th>
<th>Voges Proskauer's</th>
<th>Methyl red</th>
<th>Xylose</th>
<th>Lactose</th>
<th>Glucose</th>
<th>α-Methyl-D-Mannoside</th>
<th>Rhamnose</th>
<th>Sucrose</th>
<th>Mannitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria grayi</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Listeria innocua</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Listeria ivanovii</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Listeria ivanovii Sub sp. ivanovii</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Listeria ivanovii Sub sp. fondonensis</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Note**: Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.
+ = Positive (more than 90 %) - = Negative NR = Not Reported v = Variable reaction
## Result interpretation chart

<table>
<thead>
<tr>
<th>No.</th>
<th>Test</th>
<th>Reagents to be added after incubation</th>
<th>Principle</th>
<th>Original colour of the medium</th>
<th>Positive reaction</th>
<th>Negative reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Catalase</td>
<td>3% H₂O₂ solution</td>
<td>Detects Catalase activity</td>
<td>Colourless</td>
<td>Effervescence coming out from the loop</td>
<td>No Effervescence seen</td>
</tr>
<tr>
<td>2</td>
<td>Nitrate Reduction</td>
<td>1-2 drops Sulphanilic acid and 1-2 drops of N,N-Dimethyl-1-Naphylamine</td>
<td>Detects Nitrate reduction</td>
<td>Colourless</td>
<td>Pinkish Red</td>
<td>Colourless</td>
</tr>
<tr>
<td>3</td>
<td>Esculin Hydrolysis</td>
<td>—</td>
<td>Detects Esculin hydrolysis</td>
<td>Cream</td>
<td>Black</td>
<td>Cream</td>
</tr>
<tr>
<td>4</td>
<td>Voges-Proskauer’s test</td>
<td>1-2 drops of Barritt reagent A and 1-2 drops of Barritt reagent B</td>
<td>Detects acetoin production</td>
<td>Colourless/ Light yellow</td>
<td>Pinkish red</td>
<td>Colourless/ slight copper</td>
</tr>
<tr>
<td>5</td>
<td>Methyl red</td>
<td>1-2 drops of Methyl red reagent</td>
<td>Detects acid production</td>
<td>Colourless</td>
<td>Red</td>
<td>Yellowish-orange</td>
</tr>
<tr>
<td>6</td>
<td>Xylose</td>
<td>—</td>
<td>Carbohydrate utilization</td>
<td>Pinkish Red / Red</td>
<td>Yellow</td>
<td>Red / Pink</td>
</tr>
<tr>
<td>7</td>
<td>Lactose</td>
<td>—</td>
<td>Carbohydrate utilization</td>
<td>Pinkish Red / Red</td>
<td>Yellow</td>
<td>Red / Pink</td>
</tr>
<tr>
<td>8</td>
<td>Glucose</td>
<td>—</td>
<td>Carbohydrate utilization</td>
<td>Pinkish Red / Red</td>
<td>Yellow</td>
<td>Red / Pink</td>
</tr>
<tr>
<td>9</td>
<td>a-Methyl-D glucose</td>
<td>—</td>
<td>Carbohydrate utilization</td>
<td>Pinkish Red / Red</td>
<td>Yellow</td>
<td>Red / Pink</td>
</tr>
<tr>
<td>10</td>
<td>Mannose</td>
<td>—</td>
<td>Carbohydrate utilization</td>
<td>Pinkish Red / Red</td>
<td>Yellow</td>
<td>Red / Pink</td>
</tr>
<tr>
<td>11</td>
<td>Sucrose</td>
<td>—</td>
<td>Carbohydrate utilization</td>
<td>Triangle Red</td>
<td>Yellow</td>
<td>Orange in Red</td>
</tr>
<tr>
<td>12</td>
<td>Mannitol</td>
<td>—</td>
<td>Carbohydrate utilization</td>
<td>Pinkish Red / Red</td>
<td>Yellow</td>
<td>Red / Pink</td>
</tr>
</tbody>
</table>

### Interpretation of results
- Interpret results as per the standards given in the Result Interpretation Chart. Addition of reagents in well no 2, 4, 5, should be done at the end of incubation period that is after 24 to 48 hours.

#### Catalase Test : Well No. 1
- Scrape a loopful of growth from the surface of the 3rd well. Dip the loop in a small clean test tube with 3% H₂O₂.
- Positive catalase test is seen as effervescence coming out from the surface of the loop. No effervescence is observed in case of negative catalase test.

**Note**: 3% H₂O₂ solution has to be freshly prepared.

#### Nitrate Reduction : Well No. 2
- Add 1-2 drops of Sulphanilic acid (R015) and 1-2 drops of N,N-Dimethyl-1-Naphthylamine Reagent (R009).
- Immediate development of pinkish red colour on addition of reagent indicates positive reaction.
- No change in colour indicates negative reaction.

#### Esculin Hydrolysis : Well No.3
- Positive reaction is indicated by blackening in the 3rd well.

#### Voges-Proskauer’s Test : Well No. 4
- Add 3-4 drops of Barritt reagent A (5% α-Napthol in absolute ethanol, R029) and 1 - 2 drops of Barritt reagent B (40% Potassium hydroxide, R030).
- On addition of reagent pinkish red colour is observed within 10 minutes.
- No change in colour or a slight copper colour (due to reaction of Barritt reagent A and Barritt reagent B) denotes a negative reaction.

#### Methyl red Test : Well No. 5
- Add 1-2 drops of Methyl Red (I007) reagent.
- Reagent remains distinct red if the test is positive.
- Reagent decolourises and becomes yellow if the test is negative.

#### Carbohydrate Fermentation Test : Well No. 6 to Well No 12
- Colour of the medium changes from red colour to yellow colour due to acid production if the test is positive.
- Medium remains red in colour if the test is negative.
Important points to be taken into consideration while interpreting the result

1. Allow the reagents to come to room temperature after removal from the refrigerator.
2. In case of Carbohydrate fermentation test some microorganisms show weak reaction. In this case record the reaction as ± and incubate further for 24 hours. Orange colour after 72 hours of incubation should be interpreted as a negative reaction.
3. At times organisms give contradictory result because of mutation or the media used for isolation, cultivation and maintenance.
4. The identification index has been compiled from standard references and results of tests obtained in the laboratory.

Precautions

Clinical samples and microbial cultures should be considered potentially pathogenic and handled accordingly. Aseptic conditions should be maintained during inoculation and handling of the kits. Reagents should not come in contact with skin, eyes or clothing. 3% H₂O₂ is a extremely caustic solution, so avoid contact with skin. In case it does get on the skin, immediately flood the area with 70% Ethanol and not water, to neutralize the action.

Disposal of used material

After use, kits and the instruments used for isolation and inoculation (pipettes, loops etc.) must be disinfected using a suitable disinfectant and then discarded by incineration or autoclaving in a disposable bag.

Storage and Shelf-life

Store between 2-8°C. Shelf-life is 12 months.