In the H2S test, sodium thiosulfate is reduced to H2S which reacts with phenylpyruvate which produces a color in the presence of ferric ions. In the PD test, phenylalanine is deaminated to couples with an agente. Epidemiologists, and the food and catering industries. As high as 44% and have generated much concern among regulatory agencies, epidemiologists, and the food and catering industries. Identification of L. monocytogenes is made from pure cultures of suspect isolates by a series of conventional biochemical tests that require up to seven days for completion. MICRO-ID® Listeria is a self-contained test system of reagent impregnated paper disks that will identify the genus to species in 24 hours with accuracy, convenience, and reliability.

The genus Listeria includes the pathogen L. monocytogenes which causes human listeriosis. The occurrence of several outbreaks of food-borne listeriosis involving such foods as cole slaw, fresh salad, milk, and soft cheeses have been associated with case fatality rates as high as 44% and have generated much concern among regulatory agencies, epidemiologists, and the food and catering industries.

Identification of L. monocytogenes is made from pure cultures of suspect isolates by a series of conventional biochemical tests that require up to seven days for completion. MICRO-ID® Listeria is a self-contained test system of reagent impregnated paper disks that will identify the genus to species in 24 hours with accuracy, convenience, and reliability.

PRINCIPLE OF THE TEST

MICRO-ID® Listeria system consists of a molded, styrene tray with hinged cover which includes fifteen reaction chambers. Each reaction chamber contains filter-paper disks impregnated with reagents, which detect the presence of specific enzymes and/or metabolic end-products produced by Listeria spp. Precise quantities of reagents are applied to the disks which are arranged in a configuration that separates incompatible materials. These reagents include substrates to be acted upon by bacterial enzymes and/or detection systems that react with metabolic end-products to yield a readily identifiable color change. The first five reaction chambers contain a substrate disk and a detection disk, while the remaining ten contain a single, combination substrate/detection disk.

In the VP test, glucose and pyruvate are converted to acetyl-methylcarbinol which is oxidized to diacetyl with KOH. This forms a colored complex with arginine and α-naphthol. In the Nitrate test, nitrate is reduced to nitrite which diazotizes sulfanilic acid. This couples with an α-naphthylamine derivative to form a colored complex. In the PD test, phenylalanine is deaminated to phenylpyruvate which produces a color in the presence of ferric ions. In the H2S test, sodium thiosulfate is reduced to H2S which reacts with lead acetate to produce black lead sulfide. In the Indole test, tryptophane is metabolized to indole which forms a colored complex with α-dimethylaminobenzaldehyde. In the Ornithine test, ornithine is decarboxylated to putrescine which raises the pH of the suspension and changes the color of the indicator, bromcresol purple. Likewise, in the Lysine test, lysine is decarboxylated to cadaverine which raises the pH of the suspension and changes the color of the indicator, bromcresol purple. In the Malonate test, malonate is oxidized to alkaline products which change the color of the indicator, bromcresol purple. In the Lysine test, lysine is decarboxylated to putrescine which raises the pH of the suspension and changes the color of the indicator, bromcresol purple. In the Malonate test, malonate is oxidized to alkaline products which change the color of the indicator, bromcresol purple. In the Lysine test, lysine is decarboxylated to putrescine which raises the pH of the suspension and changes the color of the indicator, bromcresol purple.

Each MICRO-ID® Listeria tray is sealed in a foil pouch with desiccant. The tray surface is covered with clear, polypropylene tape to contain the organism suspension during incubation and allow visibility of the reaction. The inside surface of the hinged cover contains filter paper to absorb any spills resulting from handling. Identification letters for each test are printed on both the inside and outside surface of the cover. The cover contains a rectangular, roughened surface where identification information may be written.

**REAGENTS (CLASSICAL FORMULA)**

<table>
<thead>
<tr>
<th>TEST</th>
<th>ACTIVE INGREDIENTS PER TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP - Voges-Proskauer</td>
<td>Glucose  2.5 mg</td>
</tr>
<tr>
<td>(Acetoin Production)</td>
<td>Sodium Pyruvate  0.6 mg</td>
</tr>
<tr>
<td></td>
<td>Arginine  0.10 mg</td>
</tr>
<tr>
<td></td>
<td>α-Naphthol derivative  0.2 mg</td>
</tr>
<tr>
<td>N - Nitrate Reductase</td>
<td>Potassium Nitrate  0.07 mg</td>
</tr>
<tr>
<td></td>
<td>Sulfanilic Acid  0.04 mg</td>
</tr>
<tr>
<td></td>
<td>α-Naphthylamine derivative 0.03 mg</td>
</tr>
<tr>
<td>PD - Phenylalanine Deaminase</td>
<td>Phenylalanine  0.55 mg</td>
</tr>
<tr>
<td></td>
<td>Ferric chloride  0.16 mg</td>
</tr>
<tr>
<td>HS2 - Hydrogen Sulfide</td>
<td>Sodium Thioglycollate  0.31 mg</td>
</tr>
<tr>
<td></td>
<td>Lead Acetate  0.25 mg</td>
</tr>
<tr>
<td>I - Indole</td>
<td>Tryptophane  0.04 mg</td>
</tr>
<tr>
<td></td>
<td>p-Dimethylaminobenzaldehyde 0.17 mg</td>
</tr>
<tr>
<td>OD - Ornithine Decarboxylase</td>
<td>Ornithine  0.9 mg</td>
</tr>
<tr>
<td></td>
<td>Bromcresol Purple  0.004 mg</td>
</tr>
<tr>
<td>LD - Lysine Decarboxylase</td>
<td>Lysine  1.5 mg</td>
</tr>
<tr>
<td></td>
<td>Bromcresol Purple  0.004 mg</td>
</tr>
<tr>
<td>M - Malonate Utilization</td>
<td>Sodium Malonate  0.7 mg</td>
</tr>
<tr>
<td></td>
<td>Bromthymol Blue  0.009 mg</td>
</tr>
<tr>
<td>U - Urease</td>
<td>Urea  1.2 mg</td>
</tr>
<tr>
<td></td>
<td>Cresol Red  0.005 mg</td>
</tr>
<tr>
<td>E - Esculin Hydrolysis</td>
<td>Esculin  0.06 mg</td>
</tr>
<tr>
<td></td>
<td>Ferric Ammonium Citrate  0.25 mg</td>
</tr>
<tr>
<td>ONPG - α-Galactosidase</td>
<td>ONPG  0.46 mg</td>
</tr>
<tr>
<td>XYL - Xylose Fermentation</td>
<td>Xylose  0.6 mg</td>
</tr>
<tr>
<td></td>
<td>Bromcresol Purple  0.01 mg</td>
</tr>
<tr>
<td>RHAM - Rhamnose Fermentation</td>
<td>Rhamnose  2.4 mg</td>
</tr>
<tr>
<td></td>
<td>Bromcresol Purple  0.01 mg</td>
</tr>
<tr>
<td>MANN - Mannitol Fermentation</td>
<td>Mannitol  2.4 mg</td>
</tr>
<tr>
<td></td>
<td>Bromcresol Purple  0.01 mg</td>
</tr>
<tr>
<td>SORB - Sorbitol Fermentation</td>
<td>Sorbitol  2.4 mg</td>
</tr>
<tr>
<td></td>
<td>Bromcresol Purple  0.01 mg</td>
</tr>
</tbody>
</table>

*Adjusted as required to meet performance standards.

**PRECAUTIONS**

This product is for In Vitro diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

**STORAGE**

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C. Allow product to equilibrate to room temperature before use. Do not open the sealed, foil pouch until just prior to use.

**PRODUCT DETERIORATION**

This product should not be used if (1) the foil packet has been damaged or opened prior to use, (2) the expiration date has passed, or (3) there are other signs of deterioration.

**SPECIMEN COLLECTION, STORAGE, AND TRANSPORT**

Specimens should be collected and handled following recommended guidelines.
MATERIALS SUPPLIED

1. MICRO-ID® Listeria units (10)
2. Encoding forms (12)

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental culture media, (5) Quality control organisms, (6) Tryptic Soy Agar with 0.6% yeast extract (TSA-YE) and/or Tryptic Soy Agar (TSA) with 5% sheep blood (7) Gram stain reagents, (8) Oxidase test reagent (REF R36191 or R21540), (9) Catalase test reagent (3% hydrogen peroxide), (10) Sterile physiological saline (8.05% NaCl), (11) Sterile test tubes (16 x 100 mm or larger), (12) McFarland #1.0 turbidity standard or equivalent (REF R20411), (13) McFarland #1.0 turbidity standard or equivalent (REF R20411), (14) Oxidase test reagent (REF R38191 or R21540), (9) Catalase test reagent (3% hydrogen peroxide), (10) Sterile physiological saline (8.05% NaCl), (11) Sterile test tubes (16 x 100 mm or larger), (12) McFarland #1.0 turbidity standard or equivalent (REF R20411), (13) McFarland #1.0 turbidity standard or equivalent (REF R20411), (14) Rhodococcus equi (ATCC® 6939) (REF R4605400), (15) Inoculating needle, (16) Pipettes, (17) 20% Potassium Hydroxide (KOH) (REF R21231).

PROCEDURE

Inoculum Preparation:

1. Select a suspicious colony from the primary isolation plate and streak onto TSA-YE or TSA with 5% sheep blood (blood agar) to confirm purity. Incubate at 30-35°C for 24 hours or until growth is satisfactory.
2. When a pure culture is obtained, perform Gram stain, oxidase, and catalase tests. Only gram-positive, oxidase-negative, catalase-positive organisms should be tested with the MICRO-ID® Listeria system.
3. Thoroughly suspend the colonies in 4.6 ml sterile saline in a sterile test tube. The organism suspension should have a clearly visible turbidity equal to at least a McFarland #1.0 turbidity standard or equivalent. Use this inoculum for the CAMP test and for inoculation of the MICRO-ID® Listeria unit.

CAMP and β-hemolysis tests:

1. Streak β-hemolytic S. aureus and R. equi vertically onto blood agar, allowing space between streaks. Streak test strains horizontally between the vertical streaks without touching them. Incubate 24 hours at 35°C.
2. After incubation, examine plates for hemolysis in the zone of influence of the vertical S. aureus and R. equi streaks, and record results of CAMP reactions.
3. Using a colony from the purity plate, stab test strains into the agar of a dry, thickly poured blood agar plate. Also stab cultures of L. monocytogenes and L. ivanovii as positive controls and L. innocua as a negative control. Incubate for 24 hours at 35°C.
4. Examine the blood agar plates containing culture stabs using a bright light. L. monocytogenes and L. seeligeri produce a slightly cleared zone around the stab, whereas L. ivanovii produces a well-defined zone of clearing. L. innocua, L. murrayi, L. grayi, and L. welshimeri show no zone of hemolysis.

*Note: Consult appropriate references for further guidelines for CAMP and β-hemolysis tests.

MICRO-ID® Listeria Test Procedure:

1. Open the sealed, moisture-proof, foil package and place the MICRO-ID® Listeria unit flat on the bench. Do not remove the clear plastic tape covering test wells.
2. Open the cover and pipette approximately 0.3 ml of the organism suspension into each inoculation well at the top of the MICRO-ID® Listeria unit.
3. Close the cover and stand the MICRO-ID® Listeria tray upright. Ensure that the organism suspension is in contact with all substrate disks but DO NOT turn tray to moisten the detection disks.
4. Incubate upright for 24 hours at 35-37°C in ambient air (i.e., non-CO2 incubator).
5. After 4 hours, check the esculin (E) and rhamnose (RHAM) reactions (see below). If both are positive, L. monocytogenes is suspected and the strip should be incubated for an additional 20 hours. If either test is negative, the organism is not L. monocytogenes.
6. After 24 hour incubation, place the unit flat on the bench, open the lid, and add 2 drops (approximately 0.1 ml) of 20% KOH to any other inoculation well. Close the lid and hold the tray upright. Be certain that the KOH flows down into the VP test solution.
7. Rotate the MICRO-ID® Listeria unit clockwise 90° so that the upper disks in the first five wells are moistened. Hold the tray upright and tap gently on the lab bench to dislodge any suspension trapped under the upper disks.
8. Read all reactions immediately, except the VP test, as positive or negative according to the color changes listed under INTERPRETATION. Read the color of the upper disk for the first five tests; the color of the organism suspension for the remaining ten tests.
9. Allow color to develop in the VP well for approximately 10 minutes, prior to reading.
INTERPRETATION

Test | Positive Reaction | Negative Reaction
--- | --- | ---
VP | Pink to Red | Light Yellow
N | Red | Colorless to Light Pink
PD | Green | Light Yellow
H₂S | Light Brown | White
I | Pink to Red | Light Yellow to Orange
OD | Purple to Red-Purple | Amber to Yellow
LD | Purple to Red-Purple | Amber to Yellow
M | Green to Blue | Yellow
U | Orange to Red-Purple | Yellow
E | Brown to Black | No color change or Beige
ONPG | Light Yellow to Yellow | Colorless
XYL | Yellow to Amber | Red-Purple to Purple
RHAM | Yellow to Amber | Red-Purple to Purple
MANN | Yellow to Amber | Red-Purple to Purple
SORB | Yellow to Amber | Red-Purple to Purple

1. Green color in the organism suspension also indicates a positive reaction.
2. Any brown discoloration of the upper disk indicates a positive reaction. This reaction may be quite weak.

IDENTIFICATION OF ORGANISMS

1. Record test results (positive or negative) in the appropriate spaces on an Encoding Form. Note that numerical values for each positive test result are printed above the TEST RESULTS line. In each group of three tests, the numerical values for a positive reaction in the left-hand position is 4, center position 2, and right-hand position 1. The numerical value for a negative result in any position is 0.

2. Add the numerical values for each group of three tests and enter the sum in the appropriate space on the SUM OF POSITIVE VALUES line. If all tests in a group of three are negative, enter a zero as the sum.

3. Locate the octal code in the EXPECTED VALUES table. Identify the organism based on the octal code, CAMP test result, and hemolysis reaction.

EXPECTED VALUES

<table>
<thead>
<tr>
<th>Octal Code</th>
<th>CAMP Reaction</th>
<th>Hemolysis Reaction</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>R. equi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44040</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44041</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44042</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44043</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44044</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>44044</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44045</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>44045</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44046</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44047</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44050</td>
<td>+(–)²</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>44050</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44050</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>44051</td>
<td>+(–)²</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>44051</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44051</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>44054</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44055</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44055</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>44064</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>44065</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

²Note: CAMP reactions for L. seeligeri may be quite weak. If the hemolytic reaction on blood is present, the CAMP test is negative, and the octal codes are 44050 or 44051, the organism is L. seeligeri.

Sample Encoding Form

**MICRO-ID® LISTERIA ENCODING FORM**

See Package Insert for Detailed Instructions.

<table>
<thead>
<tr>
<th>TESTS</th>
<th>VP</th>
<th>N</th>
<th>PD</th>
<th>H₂S</th>
<th>I</th>
<th>OD</th>
<th>LD</th>
<th>M</th>
<th>U</th>
<th>E</th>
<th>ONPG</th>
<th>XYL</th>
<th>RHAM</th>
<th>MANN</th>
<th>SORB</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUMERICAL Value of Positive Results</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TEST RESULTS

| SUM OF POSITIVE VALUES (in each group of three reactions) |
| --- | --- | --- | --- |

ORGANISM IDENTIFICATION:

Comments:
QUALITY CONTROL
All lot numbers of MICRO-ID® Listeria have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient/sample results should not be reported.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>VP</th>
<th>N</th>
<th>PD</th>
<th>H₂S</th>
<th>I</th>
<th>OD</th>
<th>LD</th>
<th>M</th>
<th>U</th>
<th>E</th>
<th>ONPG</th>
<th>XYL</th>
<th>RHAM</th>
<th>MANN</th>
<th>SORB</th>
<th>Octal Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria monocytogenes</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>
<td>-</td>
<td>-</td>
<td>44044</td>
<td></td>
</tr>
<tr>
<td>ATCC® 19111</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>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Listeria seeligeri</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>
<td>-</td>
<td>V</td>
<td>44050</td>
<td></td>
</tr>
<tr>
<td>ATCC® 35967</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>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>
<td>+</td>
<td>+</td>
<td>44043</td>
<td></td>
</tr>
<tr>
<td>ATCC® 25400</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>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactococcus lactis subsp. cremoris</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>
<td>**</td>
<td>**</td>
<td>**</td>
<td>44044</td>
</tr>
<tr>
<td>ATCC® 19257</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>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus mitis</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>
<td>*</td>
<td>*</td>
<td>*</td>
<td>44044</td>
</tr>
<tr>
<td>ATCC® 6249</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>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Not applicable.

BIBLIOGRAPHY

PACKAGING
REF R38370, MICRO-ID® LISTERIA .............................. 10 Tests/Kit

<table>
<thead>
<tr>
<th>Symbol Legend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>REF</strong></td>
</tr>
<tr>
<td><strong>IVD</strong></td>
</tr>
<tr>
<td><strong>LAB</strong></td>
</tr>
<tr>
<td><strong>IFU</strong></td>
</tr>
<tr>
<td><strong>LOT</strong></td>
</tr>
<tr>
<td><strong>Use By</strong></td>
</tr>
</tbody>
</table>

MICRO-ID® is a registered trademark of Thermo Fisher Scientific and its subsidiaries.
ATCC® is a registered trademark of American Type Culture Collection.