Listeria Identification Kit

Intended Use
A panel of 12 tests for identification of *Listeria* species (Kit contains sterile medium for Esculin Hydrolysis, Voges Proskauer Test, Nitrate Reduction Test, Methyl Red Test, Catalase Detection and 7 different carbohydrates-Glucose, Xylose, Lactose, Mannitol, Rhamnose, α-Methyl-D-Mannoside, Ribose).

Summary
Listeria are small Gram-positive bacilli occurring worldwide in soil, water, throughout the food chain. They colonize a wide range of wild and domestic animals and man and may cause Listeriosis. The kit is used for screening food samples and clinical samples and also for validating known strains. The complete list of organisms that can be identified with this kit is given in the identification index.

Principle
Microxpress® Listeria Identification Kit is a standardized identification system, comprising 12 miniature biochemical tests for identification of *Listeria* species. This kit contains sterile media for colorimetric identification using biochemical test and carbohydrate utilization tests based on principle of pH change and substrate utilization designed to identify various metabolic properties of different bacterial species. On incubation for an appropriate period, the media are examined for colour change. The results of these tests are then compared to known standards to confirm its identification.

Kit Contents
1. 1 Kit of Listeria Identification Kit
2. Technical Product Insert with Result Interpretation Chart, Result Entry Datasheet and Identification Index
3. Nitrite Detection Strip for Nitrate Reduction Test
4. Zinc dust (ZnD) for Nitrate Reduction Test
5. Methyl Red Indicator (MR) for Methyl Red Test
6. Barritt Reagent A (B-A) for Voges Proskauer Test
7. Barritt Reagent B (B-B) for Voges Proskauer Test
8. Creatine (CR) for Voges Proskauer Test

Note: Microxpress® Listeria Identification Kit contains sufficient material to perform one test.

Biochemical Tests
Microxpress® Listeria Identification Kit is a reagent set for laboratory use only.
Kit comprises of sterile test medium for:

a) Esculin Hydrolysis (V4)
b) Voges Proskauer Test (V18)
c) Nitrate Reduction Test (V10)
d) Methyl Red test (V9)
e) Catalase Detection (V3)
f) Glucose Utilization (V26)
g) Xylose Utilization (V39)
h) Lactose Utilization (V28)
i) Mannitol Utilization (V30)
j) Rhamnose Utilization (V34)
k) α-Methyl-D-Mannoside Utilization (V19)
l) Ribose Utilization (V35)

Additional Materials Required
0.9% saline, micropipettes, culture media, activated 2% glutaraldehyde solution, sterile test tubes, incubator/water bath at 37°C±2°C.

Directions

Preparation of Inoculum:

1. Isolate the organism to be identified on Soyabean Casein Digest Agar (201190210500) or Nutrient Agar (201140030500).
2. Pick up 1-3 well isolated colonies and make a homogenous suspension in 2-3 mL sterile saline.
3. Match the turbidity of this suspension to McFarland standard number 0.5.

Note: Erroneous false negative results may be obtained if the inoculum turbidity is less than McFarland standard number 0.5.

Inoculation of the Vials:

1. Bring the kit components to room temperature before testing.
2. Open the kit aseptically.
3. Inoculate each vial with 100 µL of the above-prepared inoculum by surface inoculation method.
4. Incubate at 35°C-38°C and read the result at 18-24 hours of incubation.
5. Alternatively, the kit can also be inoculated by stabbing each individual well with a loopful of inoculum.

Methyl Red Test

1. Add 1-2 drops of Methyl Red Indicator to the test vial V9.
2. Development of red colour indicates a positive test.
3. Development of yellow colour indicates a negative test.

Voges Proskauer Test

1. Add 1-2 drops of Creatine, 2-3 drops of Barritt Reagent A and 1-2 drops of Barritt Reagent B to the test vial V18.
2. Development of pinkish red colour within 5-10 minutes indicates a positive test.
3. No colour change or copper colour indicates a negative test.

Nitrate Reduction Test

1. Dip the nitrite detection strip in the test vial V10 for the solution to be just absorbed on the reaction pad.
2. Alternatively put one drop of the incubated broth on the reaction pad and observe for colour change. If no colour change is observed, add a pinch of zinc dust (addition of too much zinc dust may result in false negative reaction)
3. Formation of pink, red or violet colour upon addition of nitrite detection strip is a positive test. No colour change upon addition of a pinch of zinc dust is a positive test.

Catalase Test

1. Take a loopful (using a platinum loop) of the inoculated culture from the vial V3 and dip in 3% H_2O_2 contained in a small, clean test tube.
2. Positive test is seen as effervescence coming out of the loop.
3. No effervescence is observed in case of negative test.

Note: 3 % H_2O_2 solution has to be freshly prepared.

Identification Index

Organisms / Tests	Catalase Detection	Nitrate Reduction Test	Esculin Hydrolysis	Voges Proskauer Test	Methyl Red Test	Xylose Utilization	Lactose Utilization	Glucose Utilization	α- methyl D-mannoside Utilization	Rhamnose Utilization	Ribose Utilization	Mannitol Utilization
Listeria grayi	+	-	+	+	+	-	+	+	+	+	+	+
Listeria monocytogenes	+	-	+	+	+	-	V	+	+	+	ND	-
Listeria innocua	+	-	+	+	+	-	+	+	+	d	-	-

Organisms / Tests	Catalase Detection	Nitrate Reduction Test	Esculin Hydrolysis	Voges Proskauer Test	Methyl Red Test	Xylose Utilization	Lactose Utilization	Glucose Utilization	α- methyl D-mannoside Utilization	Rhamnose Utilization	Ribose Utilization	Mannitol Utilization
Listeria seeligeri	+	-	+	+	+	+	+	+	-	-	-	-
Listeria ivanovii	+	-	+	+	+	+	+	+	-	-	+	-
Listeria welshimeri	+	-	+	+	+	+	+	+	+	d	d	-

Key:
Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.
+: 90% or more strains are positive; -: 90% or more strains are negative; V: Variable; d: 11-89% strains are positive; ND: Not Determined

Result Interpretation Chart

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V3	Catalase Detection	3% H_2O_2 Solution	Detects catalase activity	Colourless to cream	Effervescence when treated with 3% H_2O_2	No Effervescence
V10	Nitrate Reduction Test	Nitrite Detection Strip and a pinch of Zinc dust	Detects nitrate reduction	Colourless to cream	Pinkish red	Colourless
V4	Esculin Hydrolysis	-	Detects esculin hydrolysis	Brownish yellow	Black	Cream to amber
V18	Voges Proskauer Test	1-2 drops of Creatine, 2-3 drops of Barritt reagent A and 1-2 drops of Barritt reagent B	Detects acetoin production	Colourless to cream	Pinkish red within 5-10 minutes	Colourless / slight copper
V9	Methyl Red Test	1-2 drops of Methyl Red reagent	Detects acid production	Colourless to cream	Red	Yellow
V39	Xylose Utilization	-	Detects xylose utilization	Red	Yellow	Red / Pink
V28	Lactose Utilization	-	Detects lactose utilization	Red	Yellow	Red / Pink
V26	Glucose Utilization	-	Detects glucose utilization	Red	Yellow	Red / Pink
V19	α-methyl D-mannoside Utilization	-	Detects alpha-methyl D-mannoside utilization	Red	Yellow	Red / Pink

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V34	Rhamnose Utilization	-	Detects rhamnose utilization	Red	Yellow	Red / Pink
V35	Ribose Utilization	-	Detects ribose utilization	Red	Yellow	Red / Pink
V30	Mannitol Utilization	-	Detects mannitol utilization	Red	Yellow	Red / Pink

Result Entry Data Sheet

Sample Number	V3 Catalase Detection	V10 Nitrate Reduction Test	V4 Esculin Hydrolysis	V18 Voges Proskauer Test	V9 Methyl Red Test	V39 Xylose Utilization
Sample Number	V28 Lactose Utilization	V26 Glucose Utilization	V19 α-methyl D-mannoside Utilization	V34 Rhamnose Utilization	V35 Ribose Utilization	V30 Mannitol Utilization

Interpretation of Results

1. Add the reagents in the required vials at the end of incubation period.
2. Interpret results as per the standards given in the result interpretation chart.

Remarks

1. Microxpress® Listeria Identification Kit is an *In vitro* diagnostic kit for laboratory and professional use only. Not for medicinal use.
2. This kit cannot be used directly on clinical specimens. Only pure cultures should be used to obtain optimum results.
3. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes.
4. Erroneous false negative results may be obtained if inoculum turbidity is less than McFarland standard number 0.5.
5. At times, the organism may give contradictory results because of mutation or media used for isolation, cultivation and maintenance. Results are prominent when fresh and enriched culture is used.
6. In case of carbohydrate fermentation some microorganisms may show weak reaction. Incubate further for 48 hours. Orange colour seen after 48 hours should be a negative reaction.
7. Identification index has been compiled based on standard references and results of tests obtained in the laboratory.
8. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly. Good laboratory practices and hazard precautions must be observed at all times.

Storage and Stability

1. Store the kit at 2°C-8°C. Do Not Freeze.
2. Stability of the kit is as per the expiry date mentioned on the label.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Practical Medical Microbiology, Mackie & McCartney, 13th edition 1989, Edited by J. G. Collee, J. P. Duguid.

2. Clarke P.H. And S.T. Cowan, Biochemical Methods for Bacteriology, J. Gen. Microbiol., 1952, Vol. 6: 187-197.
3. A. L. Barry and K. L. Feeney, Two quick methods for Voges-Proskauer test, Applied Microbiology, Sept. 1967, p.: 1138-1141.
4. Coblentz, L.H 1943, Rapid detection of the production of acetyl-methyl-carbinol, Am. J. Pub. Health 33:815-817.
5. Improved 18-hour Methyl Red Test, A.L. Barry, *et al.,* Applied Microbiology, Vol. 20, No. 6, Dec. 1970, p: 866-870.
6. Nonliquid Reagent For Detecting Nitrate Reduction, Anno S. Lampe, Journal Of Clinical Microbiology, Oct. 1981, Vol. 14, No. 4, P: 452-454.
7. Bergey's Manual of Determinative Bacteriology, 9th edition 1994; Edited by John G. Holt, Noel R. Krieg.
8. Murray, P. R. and *et al.,* Manual of Clinical Microbiology Vol. 1, ASM, 8th Edition, 2003.
9. Koneman. E. W and et al., Color Atlas and Textbook of Diagnostic Microbiology lippincoh, 6th Edition, 2006.
10. Bergey's Manual of Systematic Bacteriology, The Firmicutes, 2nd edition, Vol. 3.
11. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203120360001	Biochemical Identification Kit	1 Kit (1 Test)