Salmonella Identification Kit

Intended Use
A panel of 12 tests for the identification of *Salmonella* species (Kit contains sterile medium for Methyl Red Test, Voges Proskauer Test, Citrate Utilization Test, Urease Detection, H_2S Production, ONPG Test, Lysine Decarboxylase Test and 5 different carbohydrates-Arabinose, Lactose, Maltose, Sorbitol, Dulcitol).

Summary
Salmonellosis continues to be a major public health problem worldwide. Many of the cases of *Salmonella* related gastroenteritis are due to improper handling of animal products. Microxpress® Salmonella Identification Kit can be used for screening of suspected Gram-negative *Salmonella* species isolated from feces, urine, blood or any other similar clinical isolates. The complete list of microorganisms that is possible to identify with this system is given in the identification table at the end of this package insert.

Principle
Microxpress® Salmonella Identification Kit is a standardized identification system, comprising 12 miniature biochemical tests for identification of Salmonellae. 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 on the suspected organism are then compared to known standards to confirm its identification.

Kit Contents
1. 1 Kit of Salmonella Identification Kit
2. Technical Product Insert with Result Interpretation Chart, Result Entry Datasheet and Identification Index
3. Methyl Red reagent (MR) for Methyl Red Test
4. Barritt Reagent A (B-A) for Voges Proskauer Test
5. Barritt Reagent B (B-B) for Voges Proskauer Test
6. Creatine (CR) for Voges Proskauer Test

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

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

a) Methyl Red Test (V9)
b) Voges Proskauer Test (V18)
c) Citrate Utilization Test (V2)
d) Urease Detection (V17)
e) H_2S Production (V6)
f) ONPG Test (V11)
g) Lysine Decarboxylase Test (V8)
h) Arabinose Utilization (V21)
i) Lactose Utilization (V28)
j) Maltose Utilization (V29)
k) Sorbitol Utilization (V36)
l) Dulcitol Utilization (V23)

Additional Materials Required
0.9% saline, micropipettes, culture media, activated 2% glutaraldehyde solution, sterile test tubes, incubator/water bath at 35°C-37°C, sterile mineral oil.

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. Overlay test vials V8 (for Lysine Decarboxylase Test) with sterile mineral oil.
5. Incubate at 35°C-37°C and read the result at 18-24 hours of incubation.
6. Alternatively, the kit can 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 slight copper colour (due to reaction of Barritt reagent A with Barritt reagent B) indicates a negative test.

Citrate Utilization Test

Note: Incubation has to be carried out aerobically keeping the cap of the citrate vial loose.

Identification Index

Organisms / Tests Group 1 Strains	Methyl Red Test	Voges Proskauer Test	Urease Detection	H_2S Production	Citrate Utilization Test	Lysine Decarboxylase Test	ONPG Test	Lactose Utilization	Arabinose Utilization	Maltose Utilization	Sorbitol Utilization	Dulcitol Utilization
Most Serotypes	+	-	-	+	+	+	-	-	+	+	+	+
Salmonella serovar *Typhi*	+	-	-	+	-	+	-	-	-	+	+	-
Salmonella serovar *Choleraesuis*	+	-	-	d	d	+	-	-	-	+	+	-
Salmonella serovar *Paratyphi A*	+	-	-	-	-	-	-	-	+	+	+	+
Salmonella serovar *Gallinarum*	+	-	-	+	-	+	-	-	(+)	+	-	+
Salmonella serovar *Pullorum*	+	-	-	+	-	+	-	-	+	-	-	-

Salmonella serotype *Typhimurium*	+	-	-	+	+	+	-	-	+	+	+	V
Salmonella choleraesuis subspecies *arizonae*	+	-	-	+	+	+	+	V	+	+	+	V
Organisms / Tests Group 1 Strains	**Methyl Red Test**	**Voges Proskauer Test**	**Urease Detection**	**H_2S Production**	**Citrate Utilization Test**	**Lysine Decarboxylase Test**	**ONPG Test**	**Lactose Utilization**	**Arabinose Utilization**	**Maltose Utilization**	**Sorbitol Utilization**	**Dulcitol Utilization**
Salmonella choleraesuis subspecies *diarizonae*	+	-	-	+	+	+	+	V	+	+	-	-
Salmonella choleraesuis subspecies *houtenae*	+	-	-	+	+	+	-	-	d	+	+	V
Salmonella choleraesuis subspecies *indica*	+	-	-	+	V	+	V	V	+	+	-	d
Salmonella choleraesuis subspecies *salamae*	+	-	-	+	+	+	V	-	+	+	+	V
Salmonella enterica subspecies *salamae*	+	-	-	+	+	+	(-)	-	+	+	+	+
Salmonella enterica subspecies *arizonae*	+	-	-	+	+	+	+	(-)	+	+	+	-
Salmonella enterica subspecies *diarizonae*	+	-	-	+	+	+	+	(+)	+	+	+	-
Salmonella enterica subspecies *houtenae*	+	-	-	+	+	+	-	-	+	+	+	-
Salmonella bongori	+	-	-	+	+	+	+	-	+	+	+	+
Salmonella enterica subspecies *indica*	+	-	-	+	(+)	+	d	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; d: 26-75% positive; (+): 76-89% strains are positive; (-): 11-25% strains are positive; V: Variable

Result Interpretation Chart

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V9	Methyl Red Test	1-2 drops of Methyl Red indicator.	Detects acid production	Colourless to cream	Red	Yellow
V18	Voges Proskauer Test	1-2 drops of Creatine, 2-3 drops of Barritt	Detects acetoin production	Colourless to cream	Pinkish red within 5-10 minutes	Colourless / slight copper

		reagent A and 1-2 drops of Barritt reagent B				
Code	**Test**	**Reagent to be added**	**Principle**	**Original colour of medium**	**Positive reaction**	**Negative reaction**
V17	Urease Detection	-	Detects urease activity	Orangish yellow	Pink	Orangish yellow
V6	H_2S Production	-	Detects H_2S production	Orangish yellow	Black	Orangish yellow
V2	Citrate Utilization Test	-	Detects capability of organism to utilize citrate as sole carbon source	Green	Blue	Green
V8	Lysine Decarboxylase Test	-	Detects lysine decarboxylation	Reddish purple	Purple	Yellow
V11	ONPG Test	-	Detects β-galactosidase activity	Colourless to cream	Yellow	Colourless to cream
V28	Lactose Utilization	-	Detects lactose utilization	Red	Yellow	Red / Pink
V21	Arabinose Utilization	-	Detects arabinose utilization	Red	Yellow	Red / Pink
V29	Maltose Utilization	-	Detects maltose utilization	Red	Yellow	Red / Pink
V36	Sorbitol Utilization	-	Detects sorbitol utilization	Red	Yellow	Red / Pink
V23	Dulcitol Utilization	-	Detects dulcitol utilization	Red	Yellow	Red / Pink

Result Entry Data Sheet

Sample Number	V9 Methyl Red Test	V18 Voges Proskauer Test	V17 Urease Detection	V6 H_2S Production	V2 Citrate Utilization Test	V8 Lysine Decarboxylase Test
Sample Number	V11 ONPG Test	V28 Lactose Utilization	V21 Arabinose Utilization	V29 Maltose Utilization	V36 Sorbitol Utilization	V23 Dulcitol 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® Salmonella 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.
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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.
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7. Rapid and economical method for biochemical screening of stool isolates for Salmonella and Shigella species, 2004.
8. Bergey's Manual of Determinative Bacteriology, 9th edition 1994; Edited by John G. Holt, Noel R. Krieg.
9. Murray, P. R. and *et al.,* Manual of Clinical Microbiology Vol. 1, ASM, 8th Edition, 2003.
10. Koneman. E. W and et al., Color Atlas and Textbook of Diagnostic Microbiology lippincoh, 6th Edition, 2006.
11. Bergey's Manual of Systematic Bacteriology, Proteobacteria (Part B), 2nd edition, Vol. 2.
12. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203190870001	Biochemical Identification Kit	1 Kit (1 Test)