Enterobacteriaceae Identification Test Kit

Intended Use

A panel of 12 tests for differentiation of *Enterobacteriaceae* species (Kit contain sterile medium for Indole Test, Methyl Red Test, Voges Proskauer Test, Citrate Utilization Test and 8 different carbohydrates- Glucose, Adonitol, Arabinose, Lactose, Sorbitol, Mannitol, Rhamnose, Sucrose).

Summary

Gram-negative rods are the most frequently isolated bacteria from the clinical specimens. Microxpress® Enterobacteriaceae Identification Kit can be used for screening pathogenic microorganisms from enteric, urine and other relevant specimens. The complete list of organism that is possible to identify with this system is given in the identification index at the end of this package insert.

Principle

Microxpress® Enterobacteriaceae Identification Kit is a standardized identification system, comprising 12 miniature biochemical tests for identification of *Enterobacteriaceae*. 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 Enterobacteriaceae Identification Kit
2. Technical Product Insert with Result Interpretation Chart, Result Entry Datasheet and Identification Index
3. Barritt Reagent A (B-A) for Voges-Proskauer Test
4. Barritt Reagent B (B-B) for Voges-Proskauer Test
5. Creatine (CR) for Voges-Proskauer Test
6. Methyl Red Indicator (MR) for Methyl Red Test
7. Kovac's Reagent (KOV) for Indole Test

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

Biochemical Tests

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

a) Indole Test (V7)
b) Methyl Red Test (V9)
c) Voges Proskauer Test (V18)
d) Citrate Utilization Test (V2)
e) Glucose Utilization (V26)
f) Adonitol Utilization (V20)
g) Arabinose Utilization (V21)
h) Lactose Utilization (V28)
i) Sorbitol Utilization (V36)
j) Mannitol Utilization (V30)
k) Rhamnose Utilization (V34)
l) Sucrose Utilization (V37)

Additional Material Required

0.9% saline, micropipettes, culture media, activated 2% glutaraldehyde solution, sterile test tubes, incubator/water bath at 35°C-37°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. Alternatively, the kit can also be inoculated by stabbing each individual well with a loopful of inoculum.
5. Incubate at 35°C-37°C and read the result at 18-24 hours of incubation.

Indole Test

1. Add 2-3 drops of Kovac's reagent to the test vial V7.
2. Development of pinkish red coloured ring within 10 seconds indicates a positive test.
3. Reagent remains pale coloured if the test is negative.

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 reaction.

Citrate Utilization Test

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

Identification Index

Organisms / Tests	Indole Test	Methyl Red Test	Voges Proskauer Test	Citrate Utilization Test	Glucose Utilization	Adonitol Utilization	Arabinose Utilization	Lactose Utilization	Sorbitol Utilization	Mannitol Utilization	Rhamnose Utilization	Sucrose Utilization
Budvicia aquatica	-	+	-	-	+	-	(+)	(+)	-	d	+	-
Buttiauxella agrestis	-	+	-	+	+	-	+	+	-	+	+	-
Cedecea davisae	-	+	d	+	+	-	-	(-)	-	+	-	+
Cedecea neteri	-	+	d	+	+	-	-	d	+	+	-	+
Citrobacter amalonaticus	+	+	-	+	+	-	+	d	+	+	+	-

Organisms / Tests	Indole Test	Methyl Red Test	Voges Proskauer Test	Citrate Utilization Test	Glucose Utilization	Adonitol Utilization	Arabinose Utilization	Lactose Utilization	Sorbitol Utilization	Mannitol Utilization	Rhamnose Utilization	Sucrose Utilization
Citrobacter koseri (*Citrobacter diversus*)	+	+	-	+	+	+	+	d	+	+	+	d
Citrobacter freundii	d	+	-	(+)	+	-	+	(+)	+	+	+	(+)
Enterobacter aerogenes	-	-	+	+	+	+	+	+	+	+	+	+
Enterobacter amnigenus (biogroup I)	-	-	+	d	+	+	+	d	+	+	+	+
Enterobacter amnigenus (biogroup II)	-	d	+	+	+	-	+	d	+	+	+	-
Enterobacter cloacae	-	-	+	+	+	(-)	+	+	+	+	+	+
Enterobacter gergoviae	-	-	+	+	+	-	+	d	-	+	+	+
Enterobacter sakazakii	(-)	-	+	+	+	-	+	+	-	+	+	+
Escherichia coli	+	+	-	-	+	-	+	+	+	+	(+)	d
Escherichia blattae	-	+	-	d	+	-	+	-	-	-	+	-
Escherichia fergusonii	+	+	-	(-)	+	+	+	-	-	+	+	-
Escherichia hermannii	+	+	-	-	+	-	+	d	-	+	+	d
Escherichia vulneris	-	+	-	-	+	-	+	(-)	-	+	+	-
Ewingella americana	-	(+)	+	+	+	-	-	d	-	+	(-)	-
Hafnia alvei	-	d	(+)	-	+	-	+	-	-	+	+	-
Klebsiella oxytoca	+	(-)	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae subspecies *ozaenae*	-	+	-	d	+	+	+	d	d	+	d	(-)
Klebsiella pneumoniae subspecies *pneumoniae*	-	-	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae subspecies *rhinoscleromatis*	-	+	-	-	+	+	+	-	+	+	+	d
Klebsiella terrigena	-	d	+	d	+	+	+	+	+	+	+	+
Kluyvera ascorbata	+	+	-	+	+	-	+	+	d	+	+	+
Morganella morganii	+	+	-	-	+	-	-	-	-	-	-	-

Organisms / Tests	**Indole Test**	**Methyl Red Test**	**Voges Proskauer Test**	**Citrate Utilization Test**	**Glucose Utilization**	**Adonitol Utilization**	**Arabinose Utilization**	**Lactose Utilization**	**Sorbitol Utilization**	**Mannitol Utilization**	**Rhamnose Utilization**	**Sucrose Utilization**
Pantoea agglomerans	(-)	d	d	d	+	-	+	d	d	+	(+)	d
Pantoea dispersa	-	(+)	d	+	+	-	+	-	-	+	+	-
Proteus mirabilis	-	+	d	d	+	-	-	-	-	-	-	(-)
Proteus myxofaciens	-	+	+	d	+	-	-	-	-	-	-	+
Proteus penneri	-	+	-	-	+	-	-	-	-	-	-	+
Proteus vulgaris	+	+	-	(-)	+	-	-	-	-	-	-	+
Provendencia alcalifaciens	+	+	-	+	+	+	-	-	-	-	-	(-)
Provendencia rettgeri	+	+	-	+	+	+	-	-	-	+	d	(-)
Provendencia rustigianii	+	d	-	(-)	+	-	-	-	-	-	-	d
Rahnella aquatilus	-	(+)	+	+	+	-	+	+	+	+	+	+
Salmonella bongori	-	+	-	+	+	-	+	-	+	+	(+)	-
Salmonella enterica subspecies *arizonae*	-	+	-	+	+	-	+	(-)	+	+	+	-
Salmonella serovar *Choleraesuis*	-	+	-	(-)	+	-	-	-	+	+	+	-
Salmonella enterica subspecies *diarizonae*	-	+	-	+	+	-	+	(+)	+	+	+	-
Salmonella enterica subspecies *houtenae*	-	+	-	+	+	-	+	-	+	+	+	-
Salmonella enterica subspecies *indica*	-	+	-	(+)	+	-	+	(-)	-	+	+	-
Salmonella enterica subspecies *salamae*	-	+	-	+	+	-	+	-	+	+	+	-
Salmonella serovar *Paratyphi A*	-	+	-	-	+	-	+	-	+	+	+	-
Salmonella serovar *Typhi*	-	+	-	-	+	-	-	-	+	+	-	-
Serratia entomophila	-	(-)	+	+	+	-	-	-	-	+	-	+
Serratia ficaria	-	d	d	+	+	-	+	(-)	+	+	d	+
Serratia fonticola	-	+	-	+	+	+	+	+	+	+	(+)	(-)
Serratia liquefaciens	-	+	+	+	+	-	+	-	+	+	(-)	+

Organisms / Tests	Indole Test	Methyl Red Test	Voges Proskauer Test	Citrate Utilization Test	Glucose Utilization	Adonitol Utilization	Arabinose Utilization	Lactose Utilization	Sorbitol Utilization	Mannitol Utilization	Rhamnose Utilization	Sucrose Utilization
Serratia marcescens	-	(-)	+	+	+	d	-	-	+	+	-	+
Serratia plymuthica	-	+	(+)	d	+	-	+	(+)	d	+	-	+
Serratia odorifera biogroup I	d	+	d	+	+	d	+	d	+	+	+	+
Serratia odorifera biogroup II	d	d	+	+	+	d	+	+	+	+	+	-
Serratia rubidaea	-	(-)	+	+	+	+	+	+	-	+	-	+
Shigella boydii	d	+	-	-	+	-	+	-	d	+	-	-
Shigella flexneri	d	+	-	-	+	-	d	-	d	+	-	-
Shigella dysenteriae	d	+	-	-	-	-	d	-	d	+	d	-
Shigella sonnei	-	+	-	-	+	-	+	-	-	+	d	-
Yersenia enterocolitica	d	+	-	-	+	-	+	-	+	+	-	+
Yersinia frederiksenii	+	+	-	(-)	+	-	+	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% strains are positive; (-): 11-25% strains are positive; (+): 76-89% strains are positive

Result Interpretation Chart

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V7	Indole Test	2-3 drops of Kovac's reagent	Detects deamination of tryptophan	Colourless	Pinkish red coloured ring	Colourless
V9	Methyl Red Test	1-2 drops of Methyl Red indicator	Detects acid production	Colourless	Red	Yellow
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	Pinkish red within 5-10 minutes	Colourless / Slight copper
V2	Citrate Utilization Test	-	Detects capability of organism to utilize citrate as sole carbon source	Green	Blue or growth observed	Green

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V26	Glucose Utilization	-	Detects glucose utilization	Red	Yellow	Red / Pink
V20	Adonitol Utilization	-	Detects adonitol utilization	Red	Yellow	Red / Pink
V21	Arabinose Utilization	-	Detects arabinose utilization	Red	Yellow	Red / Pink
V28	Lactose Utilization	-	Detects lactose utilization	Red	Yellow	Red / Pink
V36	Sorbitol Utilization	-	Detects sorbitol utilization	Red	Yellow	Red / Pink
V30	Mannitol Utilization	-	Detects mannitol utilization	Red	Yellow	Red / Pink
V34	Rhamnose Utilization	-	Detects rhamnose utilization	Red	Yellow	Red / Pink
V37	Sucrose Utilization	-	Detects sucrose utilization	Red	Yellow	Red / Pink

Result Entry Data Sheet

Sample Number	V7 Indole Test	V9 Methyl Red Test	V18 Voges Proskauer Test	V2 Citrate Utilization Test	V26 Glucose Utilization	V20 Adonitol Utilization
Sample Number	V21 Arabinose Utilization	V28 Lactose Utilization	V36 Sorbitol Utilization	V30 Mannitol Utilization	V34 Rhamnose Utilization	V37 Sucrose 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® Enterobacteriaceae 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.
9. 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. Evaluation of two spot-indole reagent, B.L. Lawrence, P.reich and W.H.Traub 1969, Appl. Microbiol; 17:p 923-924.
6. Rapid and economical method for biochemical screening of stool isolates for Salmonella and Shigella species, 2004.
7. Improved 18-hour Methyl Red Test, A.L. Barry, *et al.,* Applied Microbiology, Vol. 20, No. 6, Dec. 1970, p: 866-870.
8. Bergey's Manual of Determinative Bacteriology, 9th edition 1994; Edited by John G. Holt, Noel R. Krieg.
9. Bergey's Manual of Systematic Bacteriology, Proteobacteria (Part B), 2nd edition, Vol. 2.
10. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203050290001	Biochemical Identification Kit	1 Kit (1 Test)