E. coli Identification Kit

Intended Use
A panel of 12 tests for identification of *E. coli* (Kit contains sterile medium for Indole Test, Methyl Red Test, Voges Proskauer Test, Citrate Utilization Test, Glucuronidase Test, Nitrate Reduction Test, ONPG Test, Lysine Decarboxylase Test and 4 different carbohydrates-Glucose, Lactose, Sucrose, Sorbitol).

Summary
Being one of the most important members of the *Enterobacteriaceae*, *E. coli* are Gram-negative, lactose fermenting organisms and are the most common cause of uncomplicated infections of lower urinary tract. They are also routinely isolated from food, feces, water and other clinical samples. The complete list of organisms that is possible to identify with this system is given in the identification table at the end of this package insert.

Principle
Microxpress® E. coli Identification Kit is a standardized identification system, comprising 12 miniature biochemical tests for identification of *E. coli*. 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 E. coli Identification Kit
2. Technical Product Insert with Result Interpretation Chart, Result Entry Data Sheet and Identification Index
3. Methyl Red Indicator (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
7. Kovac's Reagent (KOV) for Indole Test
8. Nitrite Detection Strip for Nitrate Reduction Test
9. Zinc Dust for Nitrate Reduction Test

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

Biochemical Tests
Microxpress® E. coli 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) Glucuronidase Test (V5)
f) Nitrate Reduction Test (V10)
g) ONPG Test (V11)
h) Lysine Decarboxylase Test (V8)
i) Glucose Utilization (V26)
j) Lactose Utilization (V28)
k) Sucrose Utilization (V37)
l) Sorbitol Utilization (V36)

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 also be inoculated by stabbing each individual well with a loopful of inoculum.

Indole Test

1. Add 1-2 drops of Kovac's Reagent to the test vial V7.
2. Development of pink coloured ring indicates a positive reaction.
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 Reagent A and Reagent B) 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 inoculated 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.

Citrate Utilization Test

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

Identification Index

Organisms / Tests	Methyl Red Test	Voges Proskauer Test	Citrate Utilization Test	Indole Test	Glucuronidase Test	Nitrate Reduction Test	ONPG Test	Lysine Decarboxylase Test	Lactose Utilization	Glucose Utilization	Sorbitol Utilization	Sucrose Utilization
Esherichia coli	+	-	-	+	+	+	+	+	+	+	+	+

Esherichia coli, inactive	+	-	-	(+)	-	+	d	d	(-)	+	d	(-)
Esherichia fergusonii	+	-	(-)	+	-	+	(+)	+	-	+	-	-
Esherichia hermannii	+	-	-	+	-	+	+	-	d	+	-	d
Esherichia vulneris	+	-	-	-	-	+	+	(+)	(-)	+	-	-
Esherichia blattae	+	-	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

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	Red	Yellowish orange
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
V2	Citrate Utilization Test	-	Detects capability of organism to utilize citrate as a sole carbon source	Green	Blue	Green
V7	Indole Test	2-3 drops of Kovac's reagent	Detects deamination of tryptophan	Colourless	Pink coloured ring	Colourless
V5	Glucuronidase Test	-	Detects glucuronidase activity	Colourless to cream	Blue green fluorescence under UV light*	Colourless
V10	Nitrate Reduction Test	Nitrite Detection Strip and a pinch of Zinc dust	Detects nitrate reduction	Colourless	Pinkish red	Colourless
V11	ONPG Test	-	Detects β-galactosidase activity	Colourless	Yellow	Colourless
V8	Lysine Decarboxylase Test	-	Detects lysine decarboxylation	Reddish Purple	Purple	Yellow
V28	Lactose Utilization	-	Detects lactose utilization	Red	Yellow	Red / Pink
V26	Glucose Utilization	-	Detects glucose utilization	Red	Yellow	Red / Pink
V36	Sorbitol Utilization	-	Detects sorbitol utilization	Red	Yellow	Red / Pink
V37	Sucrose Utilization	-	Detects sucrose utilization	Red	Yellow	Red / Pink

* In Glucuronidase test vial (V5), positive reaction is shown by blue green fluorescence under long wave length (340-380 nm) UV light.

Result Entry Data Sheet

Sample Number	V7 Indole Test	V9 Methyl Red Test	V18 Voges Proskauer Test	V2 Citrate Utilization Test	V5 Glucuronidase Test	V10 Nitrate Reduction Test
Sample Number	V11 ONPG Test	V8 Lysine Decarboxylase Test	V26 Glucose Utilization	V28 Lactose Utilization	V37 Sucrose Utilization	V36 Sorbitol 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® E. coli 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. In case of lysine decarboxylase test, incubation up to 48 hours may be required.
8. Identification index has been compiled based on standard references and results of tests obtained in the laboratory.
9. 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

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9. Nonliquid Reagent For Detecting Nitrate Reduction, Anno S. Lampe, Journal Of Clinical Microbiology, Oct. 1981, Vol. 14, No. 4, P: 452-454.
10. Bergey's Manual of Determinative Bacteriology, 9th edition 1994; Edited by John G. Holt, Noel R. Krieg.
11. Murray, P. R. and et al., Manual of Clinical Microbiology Vol. 1, ASM, 8th Edition, 2003.
12. Koneman. E. W and et al., Color Atlas and Textbook of Diagnostic Microbiology lippincoh, 6th Edition, 2006.
13. Bergey's Manual of Systematic Bacteriology, Proteobacteria (Part B), 2nd edition, Vol. 2.
14. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203050300001	Biochemical Identification Kit	1 Kit (1 Test)