

Gram - Negative Bacteria Identification Test Kit

Intended Use

A panel of 13 tests for identification of Gram-negative rods (Kit contains sterile medium for Lysine Decarboxylase Test, Ornithine Decarboxylase Test, Urease Detection, Phenylalanine Deaminase Test (TDA), Nitrate Reduction, H₂S Production, Oxidase Detection, Citrate Utilization Test and 5 different carbohydrates-Glucose, Adonitol, Arabinose, Lactose, Sorbitol).

Summary

Gram-negative bacteria are found in all environments on Earth that support life. They can cause many types of infections and can spread to humans in a variety of ways. Microexpress® Gram-Negative Bacteria Identification Kit can be used for screening of suspected Gram-negative species isolated from faeces, urine, blood or any other similar clinical isolates. This kit can also be used for validation of known strains. Chlortetracycline and gentamicin inhibits bacteria and saprophytic yeasts and moulds. Dermatophytes are presumptively identified based on gross morphology and the production of alkaline metabolites, which raise the pH and cause the phenol red indicator to change the colour of the medium from yellow to red.

Principle

Microexpress® Gram-Negative Bacteria Identification Kit is a standardized identification system, comprising 13 miniature biochemical tests for identification of Gram-negative rods. 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 Gram-Negative Bacteria Identification Kit
2. Technical Product Insert with Result Interpretation Chart, Result Entry Data Sheet and Identification Index
3. TDA Reagent for Phenylalanine Deaminase Test
4. Gordon McLeod Reagent for Oxidase Test
5. Nitrite Detection Strip for Nitrate Reduction Test
6. Zinc Dust for Nitrate Reduction Test

Note: Microexpress® Gram-Negative Bacteria Identification Kit contains sufficient material to perform one test.

Biochemical Tests

Microexpress® Gram-Negative Bacteria Identification Kit is a reagent set for laboratory use only.

Kit comprises of sterile test medium for:

- a) Lysine Decarboxylase Test (V8)
- b) Ornithine Decarboxylase Test (V12)
- c) Urease Detection (V17)
- d) Phenylalanine Deamination Test (TDA) (V15)
- e) Nitrate Reduction Test (V10)
- f) H₂S Production (V6)
- g) Oxidase Detection (V13)
- h) Citrate Utilization Test(V2)
- i) Glucose Utilization (V26)
- j) Adonitol Utilization (V20)
- k) Arabinose Utilization (V21)
- l) Lactose Utilization (V28)
- m) 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 inoculum by surface inoculation method.
4. Overlay test vials V8 (for Lysine Decarboxylase Test) and V12 (for Ornithine 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.

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.

Phenylalanine Deaminase Test

1. Add 2-3 drops of TDA reagent to the test vial V15.
2. Observe for appearance of green colour. The appearance of colour may take around 5 minutes.
3. Straw to yellow colour denotes a negative reaction.

Oxidase Test

1. Add 1-2 drops of Gordon McLeod reagent (oxidase reagent) to the test vial V13. (Decant the excess reagent).
2. Development of purplish blue colour within 60 seconds indicates a positive test.
3. Cream to yellow colour 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	Citrate Utilization Test	Lysine Decarboxylase Test	Ornithine Decarboxylase Test	Urease Detection	Phenylalanine Deaminase Test	Nitrate Reduction Test	H ₂ S Production	Glucose Utilization	Adonitol Utilization	Lactose Utilization	Arabinose Utilization	Sorbitol Utilization	Oxidase Detection
<i>Aeromonas caviae</i>	+	-	-	-	+	+	-	-	-	d	+	-	+

Organisms / Tests	Citrate Utilization Test	Lysine Decarboxylase Test	Ornithine Decarboxylase Test	Urease Detection	Phenylalanine Deaminase Test	Nitrate Reduction Test	H ₂ S Production	Glucose Utilization	Adonitol Utilization	Lactose Utilization	Arabinose Utilization	Sorbitol Utilization	Oxidase Detection
<i>Aeromonas eucrenophila</i>	-	-	-	-	d	+	d	d	-	d	d	-	+
<i>Aeromonas hydrophila</i>	d	+	-	-	d	+	+	+	-	d	d	-	+
<i>Aeromonas media</i>	d	-	-	-	d	+	d	-	-	d	+	-	+
<i>Aeromonas veronii</i>	+	+	+	-	+	+	d	d	-	d	-	-	+
<i>Budvicia aquatica</i>	-	-	-	d	-	+	(+)	+	-	(+)	(+)	-	-
<i>Buttiauxella agrestis</i>	+	-	+	-	-	+	-	+	-	+	+	-	-
<i>Cedecea davisae</i>	+	-	+	-	-	+	-	+	-	(-)	-	-	-
<i>Cedecea lapagei</i>	+	-	-	-	-	+	-	+	-	d	-	-	-
<i>Cedecea neteri</i>	+	-	-	-	-	+	-	+	-	-	-	+	-
<i>Citrobacter amalonaticus</i>	+	-	+	(+)	-	+	-	+	-	+	+	+	-
<i>Citrobacter koseri</i> (C. diversus)	+	-	+	d	-	+	-	+	+	+	d	+	-
<i>Citrobacter freundii</i>	(+)	-	-	d	-	+	d	+	-	+	(+)	+	-
<i>Enterobacter aerogenes</i>	+	+	+	-	-	+	-	+	+	+	+	+	-
<i>Enterogenes amnigenus</i> biogroup 1	d	-	d	-	-	+	-	+	+	d	+	-	-
<i>Enterogenes amnigenus</i> biogroup 2	+	-	+	-	-	+	-	+	-	d	+	+	-
<i>Enterobacter sakazaki</i>	+	-	+	-	d	+	-	+	-	+	+	-	-
<i>Enterobacter taylorae</i> (E. cancerogenus)	+	-	+	-	-	+	-	+	-	-	+	-	-
<i>Enterobacter cloacae</i>	+	-	+	d	-	+	-	+	(-)	+	+	+	-
<i>Enterobacter gergoviae</i>	+	+	+	+	-	+	-	+	-	d	+	-	-
<i>Escherichia coli</i>	-	+	d	-	-	+	-	+	-	+	+	+	-
<i>Escherichia blattae</i>	d	+	+	-	-	+	-	+	-	-	+	-	-
<i>Escherichia fergusonii</i>	(-)	+	+	-	-	+	-	+	+	-	+	-	-
<i>Escherichia hermannii</i>	-	-	+	-	-	+	-	+	-	d	+	-	-
<i>Escherichia vulneris</i>	-	(+)	-	-	-	+	-	+	-	(-)	+	-	-
<i>Ewingella americana</i>	+	-	-	-	-	+	-	+	-	(-)	-	-	-
<i>Hafnia alvei</i>	-	+	+	-	-	+	-	+	-	-	+	-	-

Organisms / Tests	Citrate Utilization Test	Lysine Decarboxylase Test	Ornithine Decarboxylase Test	Urease Detection	Phenylalanine Deaminase Test	Nitrate Reduction Test	H ₂ S Production	Glucose Utilization	Adonitol Utilization	Lactose Utilization	Arabinose Utilization	Sorbitol Utilization	Oxidase Detection
<i>Klebsiella pneumoniae</i> subspecies <i>ozaenae</i>	d	d	-	-	-	(+)	-	+	+	d	+	d	-
<i>Klebsiella pneumoniae</i> subspecies <i>pneumoniae</i>	+	+	-	+	-	+	-	+	+	+	+	+	-
<i>Klebsiella pneumoniae</i> subspecies <i>rhinoscleromatis</i>	-	-	-	-	-	+	-	+	+	-	+	+	-
<i>Klebsiella terrigena</i>	d	+	(-)	-	-	+	-	+	+	+	+	+	-
<i>Kluyvera ascorbata</i>	+	+	+	-	-	+	-	+	-	+	+	d	-
<i>Morganella morganii</i>	-	-	+	+	+	+	(-)	+	-	-	-	-	-
<i>Pantoea agglomerans</i>	d	-	-	(-)	(-)	(+)	-	+	-	d	+	d	-
<i>Pantoea dispersa</i>	+	-	-	-	-	+	-	+	-	-	+	-	-
<i>Proteus mirabilis</i>	d	-	+	+	+	+	+	+	-	-	-	-	-
<i>Proteus myxofaciens</i>	d	-	-	+	+	+	-	+	-	-	-	-	-
<i>Proteus penneri</i>	-	-	-	+	+	+	d	+	-	-	-	-	-
<i>Proteus vulgaris</i>	(-)	-	-	+	+	+	+	+	-	-	-	-	-
<i>Providencia alcalifaciens</i>	+	-	-	-	+	+	-	+	+	-	-	-	-
<i>Providencia rettgeri</i>	+	-	-	+	+	+	-	+	+	-	-	-	-
<i>Providencia rustigianii</i>	(-)	-	-	-	+	+	-	+	-	-	-	-	-
<i>Pseudomonas putida</i>	ND	-	ND	V	-	-	ND	+	ND	-	ND	ND	+
<i>Pseudomonas aeruginosa</i>	ND	-	ND	V	-	+	ND	+	ND	-	ND	ND	+
<i>Pseudomonas stutzeri</i>	ND	-	ND	V	-	+	ND	+	ND	-	ND	ND	+
<i>Rahnella aquaticus</i>	+	-	-	-	+	+	-	+	-	+	+	+	-
<i>Salmonella bongori</i>	+	+	+	-	-	+	+	+	-	-	+	+	-
<i>Salmonella enteritidis</i>	+	+	+	-	-	+	+	+	-	-	+	+	-
<i>Salmonella enterica</i> subspecies <i>arizonae</i>	+	+	+	-	-	+	+	+	-	(-)	+	+	-
<i>Salmonella enterica</i>	(-)	+	+	-	-	+	d	+	-	-	-	+	-

<i>subspecies Choleraesuis</i>													
Organisms / Tests	Citrate Utilization Test	Lysine Decarboxylase Test	Ornithine Decarboxylase Test	Urease Detection	Phenylalanine Deaminase Test	Nitrate Reduction Test	H ₂ S Production	Glucose Utilization	Adonitol Utilization	Lactose Utilization	Arabinose Utilization	Sorbitol Utilization	Oxidase Detection
<i>Salmonella enterica</i> subspecies <i>diarizonae</i>	+	+	+	-	-	+	+	+	-	(+)	+	+	-
<i>Salmonella enterica</i> subspecies <i>houtenae</i>	+	+	+	-	-	+	+	+	-	-	d	+	-
<i>Salmonella enterica</i> subspecies <i>indica</i>	(+)	+	+	-	-	+	+	+	-	(-)	+	-	-
<i>Salmonella enterica</i> subspecies <i>salamae</i>	+	+	+	-	-	+	+	+	-	-	+	+	-
<i>Salmonella</i> serovar <i>Typhi</i>	-	+	-	-	-	+	+	+	-	-	-	+	-
<i>Serratia entomophila</i>	+	-	-	-	-	+	-	+	-	-	-	+	-
<i>Serratia ficaria</i>	+	-	-	-	-	+	-	+	-	(-)	+	+	-
<i>Serratia fonticola</i>	+	+	+	(-)	-	+	-	+	+	+	+	+	-
<i>Serratia marcescens</i>	+	+	+	(-)	-	+	-	+	d	-	-	+	-
<i>Serratia plymuthica</i>	d	-	-	-	-	+	-	+	-	(+)	+	d	-
<i>Serratia odorifera</i> Biogroup I	+	+	+	-	-	+	-	+	d	d	+	+	-
<i>Serratia odorifera</i> Biogroup II	+	+	-	-	-	+	-	+	d	+	+	+	-
<i>Serratia proteamaculans</i>	+	+	+	-	-	+	-	+	-	-	+	(+)	-
<i>Serratia rubidaea</i>	+	d	-	-	-	+	-	+	+	+	+	-	-
<i>Shigella boydii</i>	-	-	-	-	-	+	-	+	-	-	+	d	-
<i>Shigella flexneri</i>	-	-	-	-	-	+	-	+	-	-	d	d	-
<i>Shigella dysenteriae</i>	-	-	-	-	-	+	-	-	-	-	d	d	-
<i>Shigella sonnei</i>	-	-	+	-	-	+	-	+	-	-	+	-	-
<i>Vibrio cholerae</i>	+	+	+	-	-	+	+	-	-	-	-	-	+
<i>Vibrio cincinnatiensis</i>	(-)	+	-	-	ND	+	ND	+	-	-	-	-	+
<i>Vibrio fluvialis</i>	+	-	-	-	ND	+	ND	+	-	-	+	-	+
<i>Vibrio hollisae</i>	-	-	-	-	ND	+	ND	+	-	-	+	-	+
<i>Vibrio mimicus</i>	+	+	+	-	ND	+	ND	ND	-	-	+	-	+
<i>Vibrio vulnificus</i>	d	+	+	-	V	+	-	+	-	-	-	-	+
<i>Yersinia enterocolitica</i>	-	-	+	d	-	+	-	+	-	-	+	+	-

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<i>Yersinia intermedia</i>	-	-	+	(+)	-	+	-	+	-	d	+	+	-
<i>Yersinia pestis</i>	-	-	-	-	-	(+)	-	+	-	-	+	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: 26-75% strains are positive; (-): 11-25% strains are positive; (+): 76-89% strains are positive; ND: Not Determined

Result Interpretation Chart

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V2	Citrate Utilization Test	-	Detects capability of organism to utilize citrate as sole carbon source	Green	Blue / growth observed	Green / No growth
V8	Lysine Decarboxylase Test	-	Detects lysine decarboxylation	Reddish purple	Purple	Yellow
V12	Ornithine Decarboxylase Test	-	Detects ornithine decarboxylation	Reddish purple	Purple	Yellow
V17	Urease Detection	-	Detects urease activity	Orangish yellow	Pink	Orangish yellow
V15	Phenylalanine Deaminase Test	2-3 drops of TDA reagent	Detects phenylalanine deamination activity	Colourless to cream	Green	Straw to yellow
V10	Nitrate Reduction Test	Nitrite detection strip and a pinch of zinc dust	Detects nitrate reduction	Colourless to cream	Pinkish red	Colourless to cream
V6	H ₂ S Production	-	Detects H ₂ S production	Orangish yellow	Black	Orangish yellow
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

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V36	Sorbitol Utilization	-	Detects sorbitol utilization	Red	Yellow	Red / Pink
V13	Oxidase Detection	1-2 drops of Gordon McLeod reagent	Detects oxidase activity	Colourless to cream	Purplish blue	Colourless to Cream

Result Entry Data Sheet

Sample Number	V2 Citrate Utilization Test		V8 Lysine Decarboxylase Test		V12 Ornithine Decarboxylase Test		V17 Urease Detection		V15 Phenylalanine Deaminase Test		V10 Nitrate Reduction Test			
Sample Number	V6 H ₂ S Production		V26 Glucose Utilization		V20 Adonitol Utilization		V28 Lactose Utilization		V21 Arabinose Utilization		V36 Sorbitol Utilization		V13 Oxidase Detection	

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. Microexpress® Gram-Negative Bacteria 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

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9. Koneman. E. W and *et al.*, Color Atlas and Textbook of Diagnostic Microbiology lippincott, 6th Edition, 2006.
10. Bergey's Manual of Systematic Bacteriology, Proteobacteria (Part B), 2nd edition, Vol. 2.
11. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203070140001	Biochemical Identification Kit	1 Kit (1 Test)

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.
