

Vibrio Identification Kit

Intended Use

A panel of 20 tests for identification of *Vibrio* species (Kit contains sterile medium for Indole Test, Voges Proskauer Test, ONPG Utilization, Citrate Utilization Test, Urease Detection, Nitrite Detection, Ornithine Decarboxylase Test, Lysine Decarboxylase Test, Arginine Dihydrolase Test, 0% Salt Tolerance Test, 3% Salt Tolerance Test, 6% Salt Tolerance Test, 8% Salt Tolerance Test, 10% Salt Tolerance Test and 6 different carbohydrates-Amygdalin, Glucosamine, Sucrose, Arabinose, Mannitol, Melibiose).

Summary

Vibrio species are motile, curved or comma-shaped bacilli and have a single polar flagellum with sheet proteins. They are often found in open water, freshwater and saltwater. *Vibrio* species are facultative aerobe and Gram-negative bacterium and do not form spores. The metabolism can be oxidative and fermentative. They can be differentiated from enteric bacteria by oxidase-positive reaction and motility. Most *Vibrio* species are not fastidious and a simple C-source like glucose serves as an energy source. As it is a typically marine organism, most species require 2-4% NaCl or other salts and trace elements present in sea water for optimal growth. All identification tests should ideally be performed from non-selective agar. The oxidase test may give false negative results if performed from TCBS agar.

Principle

Micropress® Vibrio Identification 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 Vibrio 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. Kovac's Reagent (KOV) for Indole Test
6. Zinc Dust for Nitrate Reduction Test

Note: Micropress® Vibrio Identification Kit contains sufficient material to perform one test.

Biochemical Tests

Micropress® Vibrio Identification Kit is a reagent set for laboratory use only.

Kit comprises of sterile test medium for:

- a) Indole Test (V7)
- b) Voges Proskauer (V18)
- c) Citrate Utilization Test (V2)
- d) Urease Detection (V17)
- e) Nitrite Detection Test (V10)
- f) ONPG Test (V11)
- g) Ornithine Decarboxylase Test (V12)
- h) Lysine Decarboxylase Test (V8)
- i) Arginine Dihydrolase Test (V1)
- j) 0% Salt Tolerance Test (V42)
- k) 3% Salt Tolerance Test (V43)
- l) 6% Salt Tolerance Test (V44)
- m) 8% Salt Tolerance Test (V45)
- n) 10% Salt Tolerance Test (V46)
- o) Amygdalin Utilization (V40)

- p) Glucosamine Utilization (V41)
- q) Sucrose Utilization (V37)
- r) Arabinose Utilization (V21)
- s) Mannitol Utilization (V30)
- t) Melibiose Utilization (V32)

Additional Materials Required

0.9% saline, micropipettes, culture media, activated 2% glutaraldehyde solution, sterile test tubes, incubator 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), V12 (for Ornithine Decarboxylase Test) and V1 (for Arginine Dihydrolase 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 2-3 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.

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 (due to reaction of Reagent A and 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	Indole Test	Voges Proskauer Test	Citrate Utilization Test	Urease Detection	Nitrite Detection Test	ONPG Test	Ornithine Decarboxylase Test	Lysine Decarboxylase Test	Arginine Dihydrolase Test	0% Salt Tolerance Test	3% Salt Tolerance Test	6% Salt Tolerance Test	8% Salt Tolerance Test	10% Salt Tolerance Test	Amygdalin Utilization	Glucosamine Utilization	Sucrose Utilization	Arabinose Utilization	Mannitol Utilization	Melibiose Utilization
<i>Vibrio alginolyticus</i>	(+)	(+)	-	-	+	-	V	+	-	-	+	+	+	+	-	V	+	-	+	-

Organisms / Tests	Indole Test	Voges Proskauer Test	Citrate Utilization Test	Urease Detection	Nitrite Detection Test	ONPG Test	Ornithine Decarboxylase Test	Lysine Decarboxylase Test	Arginine Dihydrolase Test	0% Salt Tolerance Test	3% Salt Tolerance Test	6% Salt Tolerance Test	8% Salt Tolerance Test	10% Salt Tolerance Test	Amygdalin Utilization	Glucosamine Utilization	Sucrose Utilization	Arabinose Utilization	Mannitol Utilization	Melibiose Utilization
<i>Vibrio alginolyticus</i>	(+)	(+)	-	-	+	-	V	+	-	-	+	+	+	+	-	V	+	-	+	-
<i>Vibrio cholerae</i>	+	-	+	-	+	+	+	+	-	+	+	-	-	-	-	+	+	-	+	-
<i>Vibrio cincinnatiensis</i>	-	-	(-)	-	+	(+)	-	+	-	-	+	+	+	+	-	V	+	+	+	-
<i>Vibrio damsela</i>	-	(+)	-	-	+	-	-	d	+	-	+	+	d	-	-	-	(-)	-	-	-
<i>Vibrio fluvialis</i>	d	-	+	-	+	d	-	-	+	+	+	+	(+)	(+)	(+)	+	+	+	+	-
<i>Vibrio furnissii</i>	-	-	+	-	+	d	-	-	+	+	+	+	+	+	-	+	+	+	+	-
<i>Vibrio harveyi</i>	+	-	-	-	+	-	(+)	(+)	-	-	+	+	d	-	-	-	(+)	-	+	d
<i>Vibrio hollisae</i>	+	-	-	-	+	-	-	-	-	-	+	+	+	(-)	ND	ND	-	+	-	-
<i>Vibrio metschnikovii</i>	-	+	d	-	-	d	-	+	V	-	+	+	+	d	ND	ND	+	-	(+)	-
<i>Vibrio mimicus</i>	+	-	+	-	+	+	+	+	-	+	+	(+)	-	-	ND	ND	-	-	(+)	-
<i>Vibrio parahaemolyticus</i>	+	-	-	V	+	-	+	+	-	-	+	+	+	-	-	+	-	(+)	(+)	-
<i>Vibrio vulnificus</i>	+	-	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; ND: Not Determined

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 presence of indole	Colourless	Pink coloured ring	Colourless
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 a sole carbon source	Green	Blue or growth observed	Green
V17	Urease Detection	-	Detects urease activity	Orangish yellow	Pink	Orangish yellow

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V10	Nitrite Detection Test	Nitrite Detection Strip and a pinch of Zinc dust	Detects presence of nitrite	Colourless to cream	Pinkish red	Colourless to cream
V11	ONPG Test	-	Detects β -galactosidase activity	Colourless	Yellow	Colourless
V12	Ornithine Decarboxylase Test	-	Detects ornithine decarboxylation	Reddish purple	Purple	Yellow
V8	Lysine Decarboxylase Test	-	Detects lysine decarboxylation	Reddish purple	Purple	Yellow
V1	Arginine Dihydrolase Test	-	Detects arginine decarboxylation	Reddish purple	Purple	Yellow
V42	0% Salt Tolerance Test	-	Detects presence of growth	Reddish purple	Growth	Reddish purple without growth
V43	3% Salt Tolerance Test	-	Detects presence of growth	Reddish purple	Growth	Reddish purple without growth
V44	6% Salt Tolerance Test	-	Detects presence of growth	Reddish purple	Growth	Reddish purple without growth
V45	8% Salt Tolerance Test	-	Detects presence of growth	Reddish purple	Growth	Reddish purple without growth
V46	10% Salt Tolerance Test	-	Detects presence of growth	Reddish purple	Growth	Reddish purple without growth
V40	Amygdalin Utilization	-	Detects amygdalin utilization	Red	Yellow	Red / Pink
V41	Glucosamine Utilization	-	Detects glucosamine utilization	Red	Yellow	Red / Pink
V37	Sucrose Utilization	-	Detects sucrose utilization	Red	Yellow	Red / Pink

Code	Test	Reagent to be added	Principle	Original colour of medium	Positive reaction	Negative reaction
V21	Arabinose Utilization	-	Detects arabinose utilization	Red	Yellow	Red / Pink
V30	Mannitol Utilization	-	Detects mannitol utilization	Red	Yellow	Red / Pink
V32	Melibiose Utilization	-	Detects melibiose utilization	Red	Yellow	Red / Pink

Result Entry Data Sheet

Sample Number	V7 Indole Test	V18 Voges Proskauer Test	V2 Citrate Utilization Test	V17 Urease Detection	V10 Nitrite Detection Test	V11 ONPG Test	V12 Ornithine Decarboxylase Test	V8 Lysine Decarboxylase Test	V1 Arginine Dihydrolase Test	V42 0% Salt Tolerance Test
Sample Number	V43 3% Salt Tolerance Test	V44 6% Salt Tolerance Test	V45 8% Salt Tolerance Test	V46 10% Salt Tolerance Test	V40 Amygdalin Utilization	V37 Sucrose Utilization	V41 Glucosamine Utilization	V21 Arabinose Utilization	V30 Mannitol Utilization	V32 Melibiose 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. Microexpress® Vibrio 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, ornithine decarboxylase test and arginine dihydrolase 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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10. Schultz, L.M., Rutledge, J.E., Grodner, R.M., and Biede, S.L. 1984. Determination of the thermal death time of *Vibrio*.
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
203220130001	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.
