Technical Data

HiVibrio™ Identification Kit

KB007

Intended use

KB007 is a biochemical test kit, a combination of 12 tests for identification of *Vibrio* species from clinical specimen and non-clinical samples using pure isolate. *Vibrio* are Gram-negative, catalase positive straight or curved rods and are the causative agent of cholera. The complete list of organisms that can be identified with this system is given in the identification index provided with the kit.

Kit contents

1. Each kit contains 5/10/20 kits of KB007, sufficient material to perform 5/10/20 tests. (a combination of 12 tests for identification of *Vibrio* species which contains sterile media for Voges Proskauer's, Arginine utilization, Salt tolerance, ONPG, Citrate utilization, Ornithine utilization and 6 different carbohydrates utilization tests - Mannitol, Arabinose, Sucrose, Glucose, Salicin, Cellobiose).

2. Technical product insert. 3. Identification Index. 4. Baritt reagent A (R029) for VP test
5.Baritt reagent B (R030) for VP test 6. Result Interpretation Chart and Result Entry Datasheet.

Material Required but not supplied :

1.McFarland standard
2.Inoculation loops, pipettes
3.Enrichment medium / Isolation media

Directions

Preparation of inoculum :

1.Isolate the organism to be identified on a common medium like Nutrient Agar (M001) or BHI Agar (M211). Pick up a single isolated colony and inoculate in 5 ml Alkaline Peptone Water (M618) or BHI Broth (M210) and incubate at 35-37° C for 4-6 hours until the inoculum turbidity is greater than or equal 0.5 OD at 620nm. Some organisms may require more than 6 hours of incubation. In this case incubate till the inoculum turbidity reaches 0.5 OD at 620nm.

2.Alternatively, prepare the inoculum by picking 1-3 well isolated colonies and make a homogenous suspension in 2-3ml sterile saline. The density of the suspension should be 0.5 OD at 620nm.

Inoculation of the kit :

1. Open the kit aseptically. Peel off the sealing foil.
2. Inoculate each well with 50 µl of the above inoculum by surface inoculation method.
3. Alternatively, the kit can also be inoculated by stabbing each individual well with a loopful of inoculum.

Incubation :

Temperature of incubation : 35 - 37°C. Duration of incubation : 18 - 24 hours

Interpretation of results :

Interpret results as per the standards given in the identification index. Addition of reagents in well no 1 should be done at the end of incubation period that is after 18 - 24 hours.

Principle

KB007 is a standardized, colorimetric identification system utilizing twelve conventional biochemical tests. The tests are based on the principle of pH change and substrate utilization. On incubation, organisms undergo metabolic changes which are indicated as a colour change in the media that is either visible spontaneously or after addition of a reagent (1).

Type of specimen

Pure isolate from clinical specimen and non clinical sample

Specimen collection and handling

Refer direction

Warning and Precautions

In Vitro diagnostic Use. For professional use only. Read the label before opening the pack. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Aseptic conditions should be maintained during inoculation and handling of the kits. Reagents should not come in contact with skin, eyes or clothing. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Allow the reagents to come to room temperature after removal from the refrigerator.
2. In case of carbohydrate fermentation test some microorganisms show weak reaction. In this case record the reaction as ± and incubate further upto 48 hours. Orange colour after 48 hours of incubation should be interpreted as a negative reaction.
3. At times organisms give conflicting result because of mutation or the media used for isolation, cultivation and maintenance.
4. The identification index has been compiled from standard references and results of tests carried out in the laboratory.
5. Erroneous false negative results may be obtained if the inoculum turbidity is less than 0.5 OD.
6. Results are more prominent if an enriched culture is used instead of a suspension.
7. It cannot be used directly for clinical specimens. The microorganisms to be identified have to be first isolated on appropriate isolation media. Only pure cultures should be used.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Sterile white opaque strip with 12 wells containing media for 1.Voges Proskauer's, 2. Arginine utilization, 3. Salt tolerance, 4. ONPG, 5. Citrate utilization, 6. Ornithine utilization, 7. Mannitol, 8. Arabinose, 9. Sucrose, 10. Glucose, 11. Salicin, 12. Cellobiose

Quantity of medium

0.8 ml of medium in each well.

Sterility Check

Passes release criteria

Interpretation of results :

Interpret results as per the standards given in the identification index. Addition of reagents in well no 1 should be done at the end of incubation period that is after 18 - 24 hours

1.Voges-Proskaeur's Test : Well No. 1

- Add 2-3 drops of Baritt reagent A (R029) and 1 drop of Baritt reagent B (R030).
- Positive test is indicated by a development of pinkish red colour in 5 - 10 minutes.
- No colour change or a copper colour (due to reaction of Reagent A and Reagent B) indicates a negative reaction.

2.Arginine utilization : Well No. 2

- Positive test is indicated by a colour change to Purple/Dark Purple colour.
- Yellow colour change indicates a negative reaction.

3.Salt Tolerance : Well No. 3

- Positive test is indicated by a colour change to Reddish purple colour with growth.
- Reddish purple colour without growth change indicates a negative reaction.

4.ONPG : Well No. 4

- Positive test is indicated by a colour change to Yellow colour.
- Colourless / no colour change indicates a negative reaction.

5.Citrate utilization : Well No. 5

- Positive test is indicated by a colour change to Blue colour.
- Green colour indicates a negative reaction.

6.Ornithine utilization: Well No. 6

- Positive test is indicated by a colour change to Purple/Dark Purple colour.
- Yellow colour change indicates a negative reaction.

7.Carbohydrate utilization : Well No. 7-12

- Positive test is indicated by a colour change to Yellow colour.
- Red/pink colour change indicates a negative reaction.

Result Interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Voges Proskauer's	1-2 drops of Baritt reagent A and 1-2 drops of Baritt reagent B	Detects acetoin production	Colourless / light yellow	Pinkish red	Colourless / Slight copper
2	Arginine utilization	—	Detects arginine decarboxylation	Olive green Light purple	Purple / Dark Purple	Yellow
3	Salt tolerance (1%)	—	Detects presence of growth	Reddish purple	Growth	Reddish purple w/o
4	ONPG	—	Detects $-\beta$galactosidase activity	Colourless	Yellow	Colourless
5	Citrate utilization	—	Detects capability of organism to utilize citrate as a sole carbon source	Green	Blue	Green
6	Ornithine utilization	—	Detects Ornithine decarboxylation	Olive green to Light purple	Purple/ Dark Purple	Yellow
7	Mannitol	—	Mannitol utilization	Pinkish Red-Red	Yellow	Red / Pink
8	Arabinose	—	Arabinose utilization	Pinkish Red-Red	Yellow	Red / Pink
9	Sucrose	—	Sucrose utilization	Pinkish Red-Red	Yellow	Red / Pink
10	Glucose	—	Glucose utilization	Pinkish Red-Red	Yellow	Red / Pink
11	Salicin	—	Salicin utilization	Pinkish Red-Red	Yellow	Red / Pink
12	Cellobiose	—	Cellobiose utilization	Pinkish Red-Red	Yellow	Red / Pink

Result Entry Datasheet

No.	Test	1	2	3	4	5	6	7	8	9	10
1	Voges Proskauer's										
2	Arginine utilization										
3	Salt tolerance(1%)										
4	ONPG										
5	Citrate utilization										
6	Ornithine utilization										
7	Mannitol										
8	Arabinose										
9	Sucrose										
10	Glucose										
11	Salicin										
12	Cellobiose										

KB007 HiVibrio™ Identification Kit

Identification Index

Tests	Voges Proskauer's	Arginine utilization	Salt tolerance (1%)	ONPG	Citrate utilization	Ornithine utilization	Mannitol	Arabinose	Sucrose	Glucose	Salicin	Cellobiose
V. aestuarianus	V	-	+	-	+	V	+	-	+	-	-	nd
V. alginolyticus	+	-	+	-	+	V	+	-	+	+	-	-
V. campbellii	-	-	+	nd	V	-	V	-	-	-	-	V
V. carchariae	V	-	+	-	-	-	V	-	V	V	-	V
V. cholerae	+	-	+	+	V	+	+	-	+	+	-	-
V. cincinnatiensis	-	-	+	V	+	-	+	+	+	+	+	+
V. diazotrophicus	-	+	+	nd	+	-	+	+	+	-	+	+
V. fischeri	-	-	V	nd	V	-	+	-	-	-	V	+
V. fluvialis	-	+	+	V	+	-	+	+	+	+	-	V
V. furnissii	-	+	+	V	+	-	+	+	+	+	-	V
V. gazogenes	-	-	V	nd	+	-	+	+	+	+	+	+
V. harveyi	V	-	+	-	+	-	V	-	V	V	-	V
V. hollisae	-	-	-	-	-	-	-	+	-	+	-	-
V. logei	-	-	-	nd	-	-	+	-	-	+	-	+
V. mediterranei	-	-	+	nd	nd	-	+	-	+	+	-	+
V. metschnikovii	+	V	+	V	V	-	+	-	+	+	-	-
V. mimicus	-	-	+	+	+	+	+	-	-	+	-	-
V. natriegens	-	-	+	nd	+	+	+	+	+	+	+	V
V. nereis	-	+	+	nd	+	+	-	-	+	+	-	-
V. nigripulchritudo	-	-	+	nd	+	-	+	-	-	+	-	+
V. ordalii	-	-	+	nd	+	-	-	-	+	+	-	-
V. orientalis	-	+	+	nd	+	-	+	-	+	+	-	+
V. parahaemolyticus	-	-	+	-	+	+	+	V	-	+	-	-
V. proteolyticus	+	+	+	nd	+	+	+	-	-	+	-	-
V. salmonicida	-	-	-	nd	nd	-	+	-	-	V	-	-
V. splendidus	-	V	V	nd	+	-	+	-	V	+	-	+
V. tubiashii	-	V	+	nd	+	-	+	-	+	+	-	+
V. vulnificus	-	-	+	V	+	V	V	-	V	+	+	+

Note : Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.
+ = Positive (more than 90%)
- = Negative (more than 90%)
V = Variable (11-89% positive) .
nd = Not detected

Storage and Shelf Life

On receipt store between 2-8°C. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

After use, kits and the instruments used for isolation and inoculation (pipettes, loops etc.) must be disinfected using a suitable disinfectant and then discarded by incineration or autoclaving in a disposal bag. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1.Bergey's Manual of Systematics of Archaea and Bacteria (BMSAB), 2015.
2.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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IVD *In vitro* diagnostic medical device

2°C 8°C **Storage temperature**

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE **CE Marking**

Do not use if package is damaged

Do not re-use

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