Technical Data

KB007 HiVibrio™ Identification Kit

Introduction

KB007 is a biochemical test kit for identification and differentiation of genus Vibrio. 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.

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.

Kit Contents

1. Each kit contains sufficient material to perform 10 tests.
2. 10 kits of KB007.
3. Technical product insert.
4. Result Interpretation Chart and Result Entry Datasheet.
5. Identification Index.
6. Baritt reagent A (R029).
7. Baritt reagent B (R030).

Instructions for use

Note : KB007 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.

1. Preparation of inoculum

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

Note :

- Erroneous false negative results may be obtained if the inoculum turbidity is less than 0.5 OD.
- Results are more prominent if an enriched culture is used instead of a suspension.

2. Inoculation of the kit

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

3. 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.

Voges-Prokaeur's Test : Well No. 1

- Add 2-3 drops of Baritt reagent A and 1 drop of Baritt reagent B.
- 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.

Identification Index

Tests	Voges Proskauer's	Arginine	Salt tolerance (1%)	ONPG	Citrate	Ornithine	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

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 to Light purple	Purple / Dark Purple	Yellow
3	Salt tolerance (1%)	—	Detects presence of growth	Reddish purple	Growth	Reddish purple w/o growth
4	ONPG	—	Detects β–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	—	Carbohydrate utilization	Pinkish Red-Red	Yellow	Red / Pink
8	Arabinose	—	Carbohydrate utilization	Pinkish Red-Red	Yellow	Red / Pink
9	Sucrose	—	Carbohydrate utilization	Pinkish Red-Red	Yellow	Red / Pink
10	Glucose	—	Carbohydrate utilization	Pinkish Red-Red	Yellow	Red / Pink
11	Salicin	—	Carbohydrate utilization	Pinkish Red-Red	Yellow	Red / Pink
12	Cellobiose	—	Carbohydrate utilization	Pinkish Red-Red	Yellow	Red / Pink

Important points to be taken into consideration while interpreting the result

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 up to 48 hours. Orange colour after 48 hours of incubation should be interpreted as a negative reaction.
3. In case of Lysine, Arginine, Ornithine utilization incubation up to 48 hours may be required.
4. At times organisms give conflicting result because of mutation or the media used for isolation, cultivation and maintenance.
5. The identification index has been compiled from standard references and results of tests carried out in the laboratory.

Precautions

- Clinical samples and microbial cultures should be considered potentially pathogenic and handled accordingly.
- Aseptic conditions should be maintained during inoculation and handling of the kits.
- Reagents should not come in contact with skin, eyes or clothing.

Disposal of used material

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.

Storage and Shelf-life

Store at 2-8°C. Shelf-life is 12 months.

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