

HiBacillus™ Identification Kit

KB013

Intended use

KB013 is a combination of 12 tests for identification of *Bacillus* species from clinical specimen and non clinical samples using pure isolate. It can also be used for validating known laboratory strains.

Kit contents

1. Each kit contains 5/10 /20 kits of KB013, sufficient material to perform 5/10/20 tests. Kit contains sterile media for *Bacillus* species. (Kit contains sterile media for Malonate, Voges Proskauer's, Citrate, ONPG, Nitrate reduction, Catalase, Arginine utilization and 5 different carbohydrates utilization tests - Sucrose, Mannitol, Glucose, Arabinose, Trehalose)
2. Technical product insert
3. Sulphanilic Acid 0.8% (R015) for Nitrate test
4. α - Naphthylamine Solution (R009) for Nitrate test
5. Identification Index
6. Baritt reagent A (R029) for VP test
7. Baritt reagent B (R030) for VP test
8. Result Interpretation Chart and Result Entry Datasheet

Material Required but not supplied

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

Direction

Preparation of inoculum :

- KB013 cannot be used directly on clinical or food samples. The organism to be identified has to be first isolated and purified. Pick up a single isolated colony and inoculate in 5 ml BHI (M210) and incubate at 35-37°C for 6 to 8 hours until inoculum turbidity is 0.1 OD at 620nm.

Inoculation of the kit :

1. Open the kit aseptically. Peel off the sealing foil.
2. Inoculate each well with 50 μ l 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 : 24-48 hours

Interpretation of results :

Interpret results as per the standards given in the Result Interpretation Chart. Addition of reagents in well no 2, 5, 6 should be done at the end of incubation period that is after 24 to 48 hours.

Principle

Each KB013 is a standardized, colorimetric test system based on carbohydrate utilization and other biochemical tests specific for the identification of *Bacillus* species. The tests are based on the principle of pH change and substrate utilization. *Bacillus* species on incubation exhibit metabolic changes which are indicated by a colour change in the media that can be either interpreted visually or after addition of reagent wherever required.

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 $\pm$ 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 0.1 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 Malonate, Voges Proskauer's, Citrate, ONPG, Nitrate reduction, Catalase, Arginine utilization and 5 different carbohydrates utilization tests- Sucrose, Mannitol, Glucose, Arabinose, Trehalose

Quantity of medium

0.8 ml of medium in each well.

Sterility Check

Passes release criteria

Interpretation of results :

Interpret results as per the result interpretation chart. Addition of reagents in well no 2, 5, 6 should be done at the end of incubation period that is after 24 to 48 hours.

1.Malonate utilization: Well No. 1

- Positive test is indicated by a colour change to blue colour.
- Light green or no colour change indicates a negative reaction.

Voges-Proskauer's Test: Well No. 2

- 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.

Citrate utilization: Well No. 3

- Positive test is indicated by a colour change to blue colour.
- Green or no colour change indicates a negative reaction.

ONPG : Well No. 4

- Colour change from colorless to yellow indicates positive reaction.
- No colour change indicates negative reaction

Nitrate Reduction Test : Well No. 5

- Add 1-2 drops of Sulphanilic acid (R015) and 1-2 drops of N,N-Dimethyl-1-Naphthylamine Reagent (R009).
- Immediate development of pinkish red colour on addition of reagent indicates positive reaction.
- No change in colour indicates a negative reaction.

Catalase Test : Well No. 6

- Scrape a loopful of growth from the surface. Dip the loop in a small clean test tube with 3% H₂O.
- Positive catalase test is seen as effervescence coming out from the surface of the loop.
- No effervescence is observed in case of negative catalase test. Note : 3% H₂O₂ solution has to be freshly prepared.

Arginine utilization: Well No. 7

- Colour change to Purple / Dark Purple indicates positive reaction
- No colour change or yellow colour indicates negative reaction.

Carbohydrate fermentation Test : Well No. 8-12

- Positive test is indicated by a colour change to yellow colour.
- Red or no colour change indicates a negative reaction.

Result interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of medium	Positive reaction	Negative reaction
1	Malonate	—	Malonate utilization	Bluish green	Dark Blue	Bluish green
2	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
3	Citrate	—	Citrate utilization	Light Green	Dark Blue	Light Green
4	ONPG	—	Detects Beta galactosidase	Colourless	Yellow	Colourless
5	Nitrate Reduction	1-2 drops of sulphanilic acid and 1-2 drops of N, N-Dimethyl-1-Naphthylamine	Detects Nitrate reduction	Colourless /	Pinkish Red	Colourless
6	Catalase	3% H ₂ O ₂ solution	Detects Catalase activity	Colourless	Effervescence coming out from the loop	No Effervescence seen
7	Arginine	—	Arginine utilization	Olive Green to Light Purple	Purple / Dark Purple	Yellow
8	Sucrose	—	Sucrose utilization	Pinkish Red / Red	Yellow	Red / Pink
9	Mannitol	—	Mannitol utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Glucose	—	Glucose utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Arabinose	—	Arabinose utilization	Pinkish Red / Red	Yellow	Red / Pink

Result Entry Datasheet

No	Test	1	2	3	4	5	6	7	8	9	10
1	Malonate										
2	Voges Proskauer's										
3	Citrate										
4	ONPG										
5	Nitrate Reduction										
6	Catalase										
7	Arginine										
8	Sucrose										
9	Mannitol										
10	Glucose										
11	Arabinose										
12	Trehalose										

Identification Index of various *Bacillus* species

Test	Malonate	Voges Proskauer's	Citrate	ONPG	Nitrate Reduction	Catalase	Arginine	Sucrose	Mannitol	Glucose	Arabinose	Trehalose
<i>B. cereus</i>	-	+	+	-	D	+	D	D	—	+	-	+
<i>B. pumilis</i>	-	+	+	+	-	+	-	+	+	+	+	+
<i>B. subtilis</i>	-	+	+	+	+	+	-	+	+	+	+	+
<i>B. coagulans</i>	-	D	-	D	-	+	D	D	—	+	D	+
<i>B. atrophaeus</i>	-	+	+	NR	+	+	NR	+	+	+	+	+
<i>B. megaterium</i>	+	-	+	+	D	+	-	+	+	+	+	+
<i>B. thuringensis</i>	NR	+	+	-	+	+	+	D	—	+	-	+

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 clinical 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.

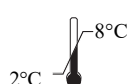
Revision:01/2023



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**In vitro diagnostic
medical device**



Storage temperature



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CE Marking



**Do not use if
package is damaged**



Do not re-use

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