Technical Data

HiAssorted™ Biochemical Test Kit

KB002

Intended use

KB002 is a combination of 12 tests for identification of Gram-negative rods. These organisms are usually oxidase negative, nitrate positive rods and are the most frequently isolated bacteria from clinical specimen and non clinical samples using pure isolate.

Kit contents

1. Each kit contains 5/10/20 kits of KB002, sufficient material to perform 5/10/20 tests. Kit contains sterile media for Citrate utilization, Lysine utilization and Ornithine utilization, Urease detection, Phenylalanine Deamination Test, Nitrate reduction, H_2S production test and 5 different carbohydrates for utilization test - Glucose, Adonitol, Lactose, Arabinose, Sorbitol.

2.Technical product insert.

3. Identification Index.

4.Sulphanilic Acid 0.8% (R015) for Nitrate test

5.α - Naphthylamine Solution (R009) for Nitrate test

6. TDA Reagent (R036) for Phenylalanine deamination

7. 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 :

1. Isolate the organism to be identified on a common medium like Nutrient Agar (M001) or Tryptone Soya Agar (M290).
2. Pick up a single isolated colony and inoculate in 5 ml BHI Broth (M210) and incubate at 35- 37°C for 4-6 hours until the inoculum turbidity is 0.1 OD at 620nm or 0.5 McFarland standard. Some organisms may require more than 6 hours of incubation. In this case incubate till the inoculum turbidity reaches 0.1 OD at 620nm.
3. 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 0.1 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 5 and 6 should be done at the end of incubation period that is after 18 - 24 hours.

Principle

KB002 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 0.1 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 1. Citrate utilization, 2. Lysine utilization, 3.Ornithine utilization, 4.Urease detection, 5. Phenylalanine deamination (TDA), 6. Nitrate reduction, 7. H_2S production test and 5 different carbohydrates for utilization test - 8. Glucose, 9. Adonitol, 10. Lactose, 11. Arabinose and 12. Sorbitol.

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 5 and 6 should be done at the end of incubation period that is after 18 - 24 hours.

1.Citrate Test : Well No. 1

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

2.Lysine utilization: Well No. 2

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

3.Ornithine utilization: Well No. 3

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

4.Urease Test: Well No. 4

- Colour changes to pink indicates positive reaction.
- No colour change indicates negative reaction.

5.Phenylalanine Deamination Test : Well No. 5

- Add 2-3 drops of TDA reagent (R036).
- Development of dark green colour within one minute indicates a positive reaction.
- No change in colour indicates negative reaction.

6.Nitrate Reduction Test : Well No. 6

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

7.H_2S production: Well No. 7

- Orangish yellow colour to black indicates a positive reaction
- No color change or slight yellowish brown indicates a negative reaction.

8.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 the medium	Positive reaction	Negative reaction
1	Citrate utilization	—	Detects capability of organism to utilize citrate as a sole carbon source	Green	Blue	Green
2	Lysine utilization	—	Detects Lysine decarboxylation	Olive green to Light Purple	Purple / Dark Purple	Yellow
3	Ornithine utilization	—	Detects Ornithine decarboxylation	Olive green to Light Purple	Purple / Dark Purple	Yellow
4	Urease	—	Detects Urease activity	Orangish yellow	Pink	Orangish yellow
5	Phenylalanine Deamination	2-3 drops of TDA reagent	Detects Phenylalanine deamination activity	Colourless	Green	Colourless
6	Nitrate reduction	1-2 drops of sulphanilic acid and 1-2 drops of N, N-Dimethyl-1-Napthylamine	Detects Nitrate reduction	Colourless	Pinkish Red	Colourless
7	H_2S production	—	Detects H_2S production	Orangish yellow	Black	Orangish yellow
8	Glucose	—	Glucose utilization	Pinkish Red / Red	Yellow	Red / Pink
9	Adonitol	—	Adonitol utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Lactose	—	Lactose utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Arabinose	—	Arabinose utilization	Pinkish Red / Red	Yellow	Red / Pink
12	Sorbitol	—	Sorbitol utilization	Pinkish Red / Red	Yellow	Red / Pink

Result Entry Datasheet

No.	Test	1	2	3	4	5	6	7	8	9	10
1	Citrate utilization										
2	Lysine utilization										
3	Ornithine utilization										
4	Urease										
5	Phenylalanine Deamination										
6	Nitrate reduction										
7	H_2S production										
8	Glucose										
9	Adonitol										
10	Lactose										
11	Arabinose										
12	Sorbitol										

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Identification Index for Gram-negative rods

Tests	Citrate utilization	Lysine	Ornithine	Urease	TDA	Nitrate reduction	H_2S production	Glucose	Adonitol	Lactose	Arabinose	Sorbitol
Aeromonas caviae	v	–	–	–	–	+	–	+	–	v	+	–
Aeromonas eucrenophila	–	–	–	–	v	+	–	+	–	–	+	–
Aeromonas hydrophila	v	v	–	–	–	+	+	+	–	v	+	–
Aeromonas media	v	–	–	–	v	+	–	+	–	v	+	–
Aeromonas veronii	+	+	+	–	v	+	–	+	–	–	–	–
Budvicia aquatica	–	–	–	v	–	+	v	+	–	v	v	–
Buttiauxella agrestis	+	–	+	–	–	+	–	+	–	+	+	–
Cedecea davisae	+	–	+	–	–	+	–	+	–	v	–	–
Cedecea lapagei	+	–	–	–	–	+	–	+	–	v	–	–
Cedecea neteri	+	–	–	–	–	+	–	+	–	v	–	+
Citrobacter amalonaticus	v	–	+	v	–	+	–	+	–	v	+	+
Citrobacter diversus	+	–	+	v	–	+	–	+	+	+	+	+
Citrobacter freundii	+	–	v	v	–	+	v	+	–	v	+	+
Enterobacter aerogenes	+	+	+	–	–	+	–	+	+	+	+	+
Enterobacter amnigenus (Biogroup I)	v	–	v	–	–	+	–	+	–	v	+	–
Enterobacter amnigenus (Biogroup II)	+	–	+	–	–	+	–	+	–	v	+	+
Enterobacter taylorae (E. cancerogenus)	+	–	+	–	–	+	–	+	–	–	+	–
Enterobacter cloacae	+	–	+	v	–	+	–	+	v	+	+	+
Enterobacter gergoviae	+	+	+	+	–	+	–	+	–	v	+	–
Enterobacter sakazakii	+	–	+	–	v	+	–	+	–	+	+	–
Escherichia coli	–	+	v	–	–	+	–	+	–	+	+	+
Escherichia coli, inactive	–	v	v	–	–	+	–	+	–	v	v	v
Escherichia blattae	v	+	+	–	–	+	–	+	–	–	+	–
Escherichia fergusonii	v	+	+	–	–	+	–	+	+	–	+	–
Escherichia hermannii	–	–	+	–	–	+	–	+	–	v	+	–
Escherichia vulneris	–	v	–	–	–	+	–	+	–	v	+	–
Ewingella americana	+	–	–	–	–	+	–	+	–	v	–	–
Hafnia alvei	–	+	+	–	–	+	–	+	–	–	+	–
Klebsiella oxytoca	+	+	–	+	–	+	–	+	+	+	+	+
Klebsiella pneumoniae subspecies *ozaenae*	v	v	–	–	–	v	–	+	+	v	+	v
Klebsiella pneumoniae subspecies pneumoniae	+	+	–	+	–	+	–	+	+	+	+	+
Klebsiella pneumoniae subspecies *rhinoscleromatis*	–	–	–	–	–	+	–	+	+	–	+	+
Klebsiella terrigena	v	+	v	–	–	+	–	+	+	+	+	+
Kluyvera ascorbata	+	+	+	–	–	+	–	+	–	+	+	v
Leclercia adecarboxylata (Escherichia adecarboxylata)	–	–	–	v	–	+	–	+	+	+	+	–
Morganella morganii subspecies *morganii*	–	–	+	+	+	+	–	+	–	–	–	–
Morganella morganii subspecies *sibonii*	–	v	v	+	+	+	–	+	–	–	–	–
Pantoea agglomerans	+	–	v	–	+	+	–	+	–	–	+	–
Pantoea dispersa	+	–	–	–	–	+	–	+	–	–	+	–
Proteus mirabilis	v	–	+	+	+	+	+	+	–	–	–	–
Proteus myxofaciens	+	–	–	+	+	+	–	+	–	–	–	–
Proteus penneri	–	–	–	+	+	+	v	+	–	–	–	–
Proteus vulgaris	v	–	–	+	+	+	+	+	–	–	–	–
Providencia alcalifaciens	+	–	–	–	+	+	–	+	+	–	–	–
Providencia rettgeri	+	–	–	+	+	+	–	+	+	–	–	–
Providencia rustigianii	v	–	–	–	+	+	–	+	+	–	–	–
Pseudomonas aeruginosa	+	–	–	v	–	+	–	+	nd	–	–	–
Pseudomonas fluorescens	+	–	–	v	–	v	–	+	nd	v	v	–
Pseudomonas putida	+	–	–	v	–	–	–	+	nd	v	v	nd

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 = 11-89% Positive, nd = No data available.

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Identification Index for Gram-negative rods

Tests	Citrate utilization	Lysine	Ornithine	Urease	TDA	Nitrate reduction	H_2S production	Glucose	Adonitol	Lactose	Arabinose	Sorbitol
Pseudomonas veronii	nd	nd	nd	v	–	+	–	+	nd	nd	nd	nd
Pseudomonas monteilii	+	–	–	v	–	–	–	+	nd	–	nd	nd
Pseudomonas stutzeri	v	–	–	v	–	+	–	+	nd	–	–	nd
Pseudomonas mendocina	+	–	–	v	–	+	–	+	nd	–	–	nd
Pseudomonas pseudoalcaligenes	v	–	–	–	–	+	–	–	nd	–	nd	nd
Pseudomonas alcaligenes	v	–	–	–	–	v	–	–	nd	–	nd	nd
Pseudomonas luteola	+	–	–	v	–	v	–	+	nd	–	nd	nd
Pseudomonas oryzihabitans	+	–	–	v	–	–	–	+	nd	v	nd	nd
Rahnella aquatilis	+	–	–	–	+	+	–	+	–	+	+	+
Salmonella Bongori	+	+	+	–	–	+	+	+	–	–	+	+
Salmonella Choleraesuis subspecies Arizonae	+	+	+	–	–	+	+	+	–	v	+	+
Salmonella Choleraesuis subspecies Choleraesuis	+	+	+	–	–	+	+	+	–	–	+	+
Salmonella Choleraesuis subspecies Diarizonae	+	+	+	–	–	+	+	+	–	v	+	+
Salmonella Choleraesuis subspecies Houtenae	+	+	+	–	–	+	+	+	–	–	+	+
Salmonella Choleraesuis subspecies Indica	v	+	+	–	–	+	+	+	–	v	+	–
Salmonella Choleraesuis subspecies Salamae	+	+	+	–	–	+	+	+	–	–	+	+
Salmonella Enteritidis	+	+	+	–	–	+	+	+	–	–	+	+
Salmonella Typhi	–	+	–	–	–	+	+	+	–	–	–	+
Serratia entomophila	+	–	–	–	–	+	–	+	–	–	–	–
Serratia ficaria	+	–	–	–	–	+	–	+	–	v	+	+
Serratia fonticola	+	+	+	v	–	+	–	+	+	+	+	+
Serratia marcescens	+	+	+	v	–	+	–	+	v	–	–	+
Serratia plymuthica	v	–	–	–	–	+	–	+	–	v	+	v
Serratia odorifera (Biogroup I)	+	+	+	–	–	+	–	+	v	v	+	+
Serratia odorifera (Biogroup II)	+	+	–	–	–	+	–	+	v	+	+	+
Serratia proteamaculans	+	+	+	–	–	+	–	+	–	–	+	v
Serratia rubidaea	+	v	–	–	–	+	–	+	+	+	+	–
Shigella boydii, Shigella flexneri, Shigella dysenteriae	–	–	–	–	–	+	–	+	–	–	v	v
Shigella sonnei	–	–	+	–	–	+	–	+	–	–	+	–
Vibrio alginolyticus	–	+	v	–	–	+	–	+	–	–	–	–
Vibrio cholerae	–	+	+	+	–	–	+	–	+	–	–	–
Vibrio cincinnatiensis	v	+	–	–	–	+	–	+	–	–	+	–
Vibrio fluvialis	+	–	–	–	–	+	–	+	–	–	+	–
Vibrio furnissis	+	–	–	–	–	+	–	+	–	–	+	–
Vibrio harveyi	–	+	–	–	nd	+	–	v	–	–	–	–
Vibrio hollisae	–	–	–	–	–	+	–	+	–	–	+	–
Vibrio metschnikovii	v	v	–	–	–	–	–	+	–	v	–	v
Vibrio mimicus	+	+	+	–	–	+	–	+	–	v	–	–
Vibrio parahaemolyticus	–	+	+	v	–	+	–	+	–	–	v	–
Vibrio vulnificus	v	+	v	–	v	+	–	+	–	v	–	–
Yersinia enterocolitica	–	–	+	v	–	+	–	+	–	–	+	+
Yersinia frederiksenii	v	–	+	v	–	+	–	+	–	v	+	+
Yersinia intermedia	–	–	+	v	–	+	–	+	–	v	+	+
Yersinia pestis	–	–	–	–	–	v	–	+	–	–	+	v
Yersinia pseudotuberculosis	–	–	–	+	–	+	–	+	–	–	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 = 11-89% Positive, nd = No data available.

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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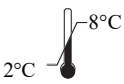
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U,Esdoornlaan13,
3951DB Maarn, NL
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CE **CE Marking**

Do not use if package is damaged

Do not re-use

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