KB003 Hi25™ *Enterobacteriaceae* Identification Kit

Introduction

KB003 is a comprehensive test system that can be used for identification of gram-negative *Enterobacteriaceae* species. Organisms belonging to *Enterobacteriaceae* are gram negative, oxidase negative, nitrate positive rods and are the most frequently isolated bacteria from clinical specimens. Hi25™ identification kit can be used for screening pathogenic organisms from urine, enteric specimens and other relevant clinical samples. It can also be used for validating known laboratory strains. The complete list of organisms that can be identified with this system is given in the identification index provided with the kit.

Principle

Each Hi25™ kit is a standardized colorimetric identification system utilizing thirteen conventional biochemical tests and eleven carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation organisms undergo metabolic changes which are indicated by a colour change in the media that is either visible spontaneously or after addition of a reagent. Oxidase test is performed separately using oxidase reagent disc provided with the kit.

Kit contents

1. Each kit contains sufficient material to perform 10 tests.
2. 10 kits of Part I.
3. 10 kits of Part II.
4. Oxidase reagent discs (DD018)
5. Technical product insert.
6. Result Interpretation Chart and Result Entry Datasheet.
7. Identification Index.
8. TDA reagent (R036) for Phenylalanine Deaminase test.
9. Baritt reagent A (R029) for Voges-Proskauer's test.
10. Baritt reagent B (R030) for Voges-Proskauer's test.
11. Methyl Red reagent (I007) for Methyl Red test
12. Kovac's reagent (R008) for Indole test
13. Sulphanilic acid (R015)
14. N,N-Dimethyl-1-Napthylamine Reagent (R009).

Instructions for use

1. **Preparation of inoculum**
   - KB003 cannot be used directly on clinical specimens. The organisms to be identified have to be first isolated and purified. Only pure cultures should be used.
   - Isolate the organism to be identified on a common medium like Nutrient Agart (M001/ M1274) or a differential medium like MacConkey Agar (M082)..
   - Pick up a single isolated colony and inoculate in 5 ml Brain Heart Infusion Broth and incubate at 35-37°C for 4-6 hours until the inoculum turbidity is ≥ 0.1OD at 620nm or 0.5 McFarland standard. Some fastidious organisms may require more than 6 hours of incubation. In this case incubate till the inoculum turbidity reaches 0.1OD 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 0.1OD at 620nm.
   - Perform Oxidase test on the organism to be tested. The test is performed using Oxidase disc (DD018) provided with the kit.
   - Pick up a well isolated colony and rub it on a single oxidase disc. Positive reaction is indicated by development of deep purple colour within 10 seconds. Colour change in 10-60 seconds indicates a delayed positive reaction. Colour development after 60 seconds or no change in colour indicates a negative reaction.
   - Note the result in the Result Entry Datasheet. Oxidase test must be performed as it is an integral part of the identification system. It must be performed to differentiate *Enterobacteriaceae* from other gram negative rods.

Note
- Erroneous false negative results may be obtained if the inoculum turbidity is less than 0.1 OD.
- Results are more prominent if an enriched culture is used instead of 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 ± 2°C. Duration of incubation: 18 - 24 hours.

Interpretation of results :

Interpret results as per the standards given in the identification index. Addition of reagents wherever required should be done at the end of incubation period that is after 18 - 24 hours.

Part I : 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 denotes a negative reaction.

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.

Voges Proskauer's Test : Well No. 9 Add 2-3 drops of Baritt reagent A (R029) and 1 drop of Baritt reagent B(R030).
- Pinkish red colour development within 5-10 minutes indicates a positive test.
- No change in colour or a slight change in colour (due to reaction of Baritt reagent A with Baritt reagent B) denotes a negative reaction.

Methyl Red Test : Well No. 10 Add 1-2 drops of Methyl Red reagent (I007).
- Reagent remains red in colour if the test is positive.
- Reagent decolourises and becomes yellow if the test is negative.

Indole Test : Well No. 11 Add 1-2 drops of Kovac's reagent (R008).
- Development of pinkish red colour within 10 seconds indicates positive reaction.
- Reagent remains pale coloured if the test is negative.

Identification Index of various *Enterobacteriaceae species*

Tests	ONPG	Lysine	Ornithine	Urease	TDA	Nitrate	H_2S	Citrate Utilization	Voges Proskauer's	Methyl Red	Indole	Malonate
Budvicia aquatica	+	–	–	V	–	+	V	–	–	+	–	–
Buttiauxella agrestis	+	–	+	–	–	+	–	+	–	+	–	V
Cedecea davisae	+	–	+	–	–	+	–	+	V	+	–	+
Cedecea lapagei	+	–	–	–	–	+	–	+	V	V	–	+
Cedecea neteri	+	–	–	–	–	+	–	+	V	+	–	+
Citrobacter amalonaticus	+	–	+	V	–	+	–	V	–	+	+	–
Citrobacter diversus	+	–	+	V	–	+	–	+	–	+	+	+
Citrobacter freundii	+	–	V	V	–	+	V	+	–	+	–	V
Enterobacter aerogenes	+	+	+	–	–	+	–	+	+	–	–	+
Enterobacter amnigenus (Biogroup I)	+	–	V	–	–	+	–	V	+	–	–	+
Enterobacter amnigenus (Biogroup II)	+	–	+	–	–	+	–	+	+	V	–	+
Enterobacter taylorae (E. cancerogenus)	+	–	+	–	–	+	–	+	+	–	–	+
Enterobacter cloacae	+	–	+	V	–	+	–	+	+	–	–	V
Enterobacter gergoviae	+	+	+	+	–	+	–	+	+	–	–	+
Enterobacter sakazakii	+	–	+	–	V	+	–	+	+	–	V	V
Escherichia coli	+	+	V	–	–	+	–	–	–	+	+	–
Escherichia coli, inactive	V	V	V	–	–	+	–	–	–	+	V	–
Escherichia blattae	–	+	+	–	–	+	–	V	–	+	–	+
Escherichia fergusonii	V	+	+	–	–	+	–	V	–	+	+	V
Escherichia hermannii	+	–	+	–	–	+	–	–	–	+	+	–
Escherichia vulneris	+	V	–	–	–	+	–	–	–	+	–	V
Ewingella americana	V	–	–	–	–	+	–	+	+	V	–	–
Hafnia alvei	+	+	+	–	–	+	–	–	V	V	–	V
Klebsiella oxytoca	+	+	–	+	–	+	–	+	+	V	+	+
Klebsiella pneumoniae subspecies ozaenae	V	V	–	–	–	V	–	V	–	+	–	–
Klebsiella pneumoniae subspecies pneumoniae	+	+	–	+	–	+	–	+	+	V	–	+
Klebsiella pneumoniae subspecies rhinoscleromatis	–	–	–	–	–	+	–	–	–	+	–	+
Klebsiella terrigena	+	+	V	–	–	+	–	V	+	V	–	+
Kluyvera ascorbata	+	+	+	–	–	+	–	+	–	+	+	+
Leclercia adecarboxylata (Escherichia adecarboxylata)	+	–	–	V	–	+	–	–	–	+	+	+
Morganella morganii subspecies morganii	–	–	+	+	+	+	–	–	–	+	+	–
Morganella morganii subspecies sibonii	–	V	V	+	+	+	–	–	–	V	V	–
Pantoea agglomerans	+	–	V	–	V	+	–	+	+	V	–	+
Pantoea dispersa	+	–	–	–	–	V	–	+	+	V	–	–
Proteus mirabilis	–	–	+	+	+	+	+	V	V	+	–	–
Proteus myxofaciens	–	–	–	+	+	+	–	+	+	+	–	–
Proteus penneri	–	–	–	+	+	+	V	–	–	+	–	–
Proteus vulgaris	–	–	–	+	+	+	+	V	–	+	+	–
Providencia alcalifaciens	–	–	–	–	+	+	–	+	–	+	+	–
Providencia rettgeri	–	–	–	+	+	+	–	+	–	+	+	–
Providencia rustigianii	–	–	–	–	+	+	–	V	–	V	+	–
Rahnella aquatilis	+	–	–	–	+	+	–	+	+	V	–	+
Salmonella bongori	+	+	+	–	–	+	+	+	–	+	–	–
Salmonella choleraesuis subspecies arizonae	+	+	+	–	–	+	+	+	–	+	–	+
Salmonella choleraesuis subspecies choleraesuis	–	+	+	–	–	+	+	+	–	+	–	–
Salmonella choleraesuis subspecies diarizonae	+	+	+	–	–	+	+	+	–	+	–	+
Salmonella choleraesuis subspecies houtenae	–	+	+	–	–	+	+	+	–	+	–	–
Salmonella choleraesuis subspecies indica	V	+	+	–	–	+	+	V	–	+	–	–
Salmonella choleraesuis subspecies salamae	V	+	+	–	–	+	+	+	–	+	–	+
Salmonella enteritidis	–	+	+	–	–	+	+	+	–	+	–	–
Salmonella typhi	–	+	–	–	–	+	+	–	–	+	–	–
Salmonella typhimurium	–	+	+	–	–	+	+	+	–	+	–	–
Serratia entomophila	+	–	–	–	–	+	–	+	+	V	–	–
Serratia ficaria	+	–	–	–	–	+	–	+	V	V	–	–
Serratia fonticola	+	+	+	V	–	+	–	+	–	+	–	V
Serratia marcescens	+	+	+	V	–	+	–	+	+	V	–	–
Serratia odorifera (Biogroup I)	+	+	+	–	–	+	–	+	V	+	V	–
Serratia odorifera (Biogroup II)	+	+	–	–	–	+	–	+	+	V	V	–
Serratia plymuthica	V	–	–	–	–	+	–	V	V	+	–	–
Serratia proteamaculans	+	+	+	–	–	+	–	+	V	V	–	–
Serratia rubidaea	+	V	–	–	–	+	–	+	+	V	–	+
Shigella boydii, Shigella flexneri, Shigella dysenteriae	–	–	–	–	–	+	–	–	–	+	V	–
Shigella sonnei	+	–	+	–	–	+	–	–	–	+	–	–
Yersinia enterocolitica	+	–	+	V	–	+	–	–	–	+	V	–
Yersinia frederiksenii	+	–	+	V	–	+	–	V	–	+	+	–
Yersinia intermedia	+	–	+	V	–	+	–	–	–	+	+	–
Yersinia pestis	V	–	–	–	–	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.

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Identification Index of various *Enterobacteriaceae species*

Esculin hydrolysis	Arabinose	Xylose	Adonitol	Rhamnose	Cellobiose	Melibiose	Saccharose	Raffinose	Trehalose	Glucose	Lactose
−	V	+	−	+	−	−	−	−	−	+	V
+	+	+	−	+	+	+	−	+	+	+	+
V	−	+	−	−	+	−	+	−	+	+	V
+	−	−	−	−	+	−	−	−	+	+	V
+	−	+	−	−	+	−	+	−	+	+	V
−	+	+	−	+	+	−	V	−	+	+	V
−	+	+	+	+	+	−	V	−	+	+	V
−	+	+	−	+	V	V	V	V	+	+	V
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	−	+	+	+	+	+	+	+	V
+	+	+	−	+	+	+	−	−	+	+	V
+	+	+	−	+	+	−	−	−	+	+	−
+	+	+	−	+	+	+	+	+	+	+	V
V	+	+	+	V	+	+	V	+	+	+	V

+	+	+	−	+	+	+	+	+	+	+	V
+	+	+	−	+	+	+	+	+	+	+	+
V	+	+	−	V	−	V	V	V	+	+	+
−	V	V	−	V	−	V	V	V	+	+	V
−	+	+	−	+	−	−	−	−	V	+	−
V	+	+	+	+	+	−	−	−	+	+	−
V	+	+	−	+	+	−	V	V	+	+	V
V	+	+	−	+	+	+	−	+	+	+	V
V	−	V	−	V	−	−	−	−	+	+	V
−	+	+	−	+	V	−	−	−	+	+	−
+	+	+	+	+	+	+	+	+	+	+	+
V	+	+	+	V	+	+	V	+	+	+	V

+	+	+	+	+	+	+	+	+	+	+	+
V	+	+	+	+	+	+	V	+	+	+	−
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	−	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	V	V	+	+	+
−	−	−	−	−	−	−	−	−	−	+	−
+	−	−	−	−	−	−	−	−	+	+	−
+	+	+	−	+	V	−	+	−	+	+	V
−	V	+	−	+	V	−	+	−	+	+	−
−	−	+	−	−	−	−	V	−	+	+	−
−	−	−	−	−	−	−	+	−	+	+	−
−	−	+	−	−	−	−	+	−	V	+	−

V	−	+	−	−	−	−	+	−	V	+	−
−	−	−	+	−	−	−	V	−	−	+	−
V	−	−	+	V	−	−	V	−	−	+	−
−	−	−	+	−	−	−	V	−	−	+	−
+	+	+	−	+	+	+	+	+	+	+	+
−	+	+	−	+	−	V	−	−	+	+	−
−	+	+	−	+	−	+	−	−	+	+	V
−	+	+	−	+	−	+	−	−	+	+	−
−	+	+	−	+	−	+	−	−	+	+	V
−	+	+	−	+	V	+	−	−	+	+	−
−	+	+	−	+	−	V	−	−	+	+	V
V	+	+	−	+	−	−	−	−	+	+	−
−	+	+	−	+	−	+	−	−	−	+	−

−	−	V	−	−	−	+	−	−	+	+	−
−	+	+	−	+	−	+	−	−	−	+	−
+	−	V	−	−	−	−	+	−	+	+	−
+	+	+	−	V	+	V	+	V	+	+	V
+	+	V	+	V	−	+	V	+	+	+	+
+	−	−	V	−	−	−	+	−	+	+	−
+	+	+	V	+	+	+	+	+	+	+	V
V	+	+	V	+	+	+	−	−	+	+	+
V	+	+	−	−	V	+	+	+	+	+	V
V	+	+	_	V	−	+	+	+	+	+	−
+	+	+	+	−	+	+	+	+	+	+	+
−	V	−	−	−	−	V	−	V	V	+	−
−	+	−	−	V	−	V	−	−	+	+	−

V	+	V	−	−	V	−	+	−	+	+	−
V	+	+	−	+	+	−	+	V	+	+	V
+	+	+	−	+	+	V	+	V	+	+	V
V	+	+	−	−	−	V	−	−	+	+	−
+	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 = 11-89% positive.

Strip I	Result Interpretation chart					
No.	**Test**	**Reagents to be added after incubation**	**Principle**	**Original colour of the medium**	**Positive reaction**	**Negative reaction**
1	ONPG	—	Detects β – galactosidase activity	Colourless	Yellow	Colourless
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	Citrate utilization	—	Detects capability of organism to utilize citrate as a sole carbon source	Green	**Blue**	Green
9	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
10	Methyl red	1-2 drops of Methyl red reagent	Detects acid production	Colourless	Red	Yellowish- orange
11	Indole	1-2 drops of Kovac's red reagent	Detects deamination of tryptophan	Colourless	Pinkish Red	Colourless
12	Malonate utilization	—	Detects capability of organism to utilize sodium malonate as a sole carbon source	Light green	Blue	Light green

Strip II	Result Interpretation chart				
No.	**Test**	**Principle**	**Original colour of the medium**	**Positive reaction**	**Negative reaction**
13	Esculin hydrolysis	Esculin hydrolysis	Cream	**Black**	Cream
14	Arabinose	Arabinose utilization	Pinkish Red / Red	Yellow	Red / Pink
15	Xylose	Xylose utilization	Pinkish Red / Red	Yellow	Red / Pink
16	Adonitol	Adonitol utilization	Pinkish Red / Red	Yellow	Red / Pink
17	Rhamnose	Rhamnose utilization	Pinkish Red / Red	Yellow	Red / Pink
18	Cellobiose	Cellobiose utilization	Pinkish Red / Red	Yellow	Red / Pink
19	Melibiose	Melibiose utilization	Pinkish Red / Red	Yellow	Red / Pink
20	Saccharose	Saccharose utilization	Pinkish Red / Red	Yellow	Red / Pink
21	Raffinose	Raffinose utilization	Pinkish Red / Red	Yellow	Red / Pink
22	Trehalose	Trehalose utilization	Pinkish Red / Red	Yellow	Red / Pink
23	Glucose	Glucose utilization	Pinkish Red / Red	Yellow	Red / Pink
24	Lactose	Lactose utilization	Pinkish Red / Red	Yellow	Red / Pink
25	Oxidase	Done on Oxidase disc separately. Detects cytochrome oxidase production.	Colourless	**Deep purple within 10 seconds**	White/ Purple after 60 seconds

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 for 48 hours. Orange colour after 48 hours of incubation should be interpreted as a negative reaction.
3. In case of Lysine and Ornithine decarboxylation, 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 & Shelf-life

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

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