

## KB016 Hi24™ *Enterobacteriaceae* Identification Kit

### Introduction

KB016 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. Hi24™ 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 Hi24™ 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.

### Kit contents

- |   |   |
|---|---|
| 1. Each kit contains sufficient material to perform 10 tests. | 7. TDA reagent (R036) for Phenylalanine Deaminase test. |
| 2. 10 kits of Part I.   | 8. Baritt reagent A (R029) for Voges-Proskauer's test.  |
| 3. 10 kits of Part II.  | 9. Baritt reagent B (R030) for Voges-Proskauer's test.  |
| 4. Technical product insert.                                  | 10. Methyl Red reagent (I007) for Methyl Red test       |
| 5. Result Interpretation Chart and Result Entry Datasheet.    | 11. Kovac's reagent (R008) for Indole test              |
| 6. Identification Index.                                      | 12. PYR Reagent (R043)                                  |

### Instructions for use

#### 1. Preparation of inoculum

- KB016 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 Agar (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 0.1 OD 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 0.1 OD 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 0.1 OD at 620nm.

**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.

#### Voges Proskauer's Test : Well No. 6 ● 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. 7 ● 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. 8 ● 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.

#### PYR Test : Well No. 9 ● Add 1-2 drops of PYR reagent (R043).

- Positive test is indicated by development and reaction of cherry red colour.
- Development of Pink, Orange or Yellow colour indicates a negative reaction.

Tests	ONPG	Lysine	Ornithine	Urease	Phenylalanine	VP	Methyl red	Indole	PYR	B Glucuronidase	Galactosidase
<i>Citrobacter amalonaticus</i>	+	-	+	-	-	-	+	+	+	-	-
<i>Citrobacter braakii</i>	+	-	+	-	-	-	+	(-)	+	-	+
<i>Citrobacter farmeri</i>	+	-	+	-	-	-	+	+	+	-	+
<i>Citrobacter freundii</i>	+	-	-	-	-	-	+	(-)	+	-	+
<i>Citrobacter koseri</i>	+	-	+	-	-	-	+	+	+	-	-
<i>Citrobacter sedlakii</i>	+	-	+	-	-	-	+	+	+	-	+
<i>Citrobacter werkmanii</i>	+	-	-	-	-	-	+	-	+	-	(-)
<i>Citrobacter youngae</i>	+	-	-	-	-	-	+	(-)	+	-	(-)
<i>Edwardsiella hoshinae</i>	-	+	+	-	-	-	+	+	-	-	V
<i>Edwardsiella tarda</i>	-	+	+	-	-	-	+	+	-	-	V
<i>Enterobacter aerogenes</i>	+	+	+	-	-	-	+	-	+	-	+
<i>Enterobacter amnigenus</i>	+	-	+	-	-	-	V	-	+	-	V
<i>Enterobacter cancerogenus</i>	+	-	+	-	-	+	-	-	+	-	V
<i>Enterobacter cloacae</i>	+	-	+	(-)	-	+	-	-	+	-	V
<i>Enterobacter gergoviae</i>	+	+	+	+	-	+	-	-	-	-	V
<i>Enterobacter sakazakii</i>	+	-	+	-	-	+	-	(-)	+	-	V
<i>Escherichia blattae</i>	-	+	+	-	-	-	+	-	-	-	V
<i>Escherichia coli</i>	+	+	V	-	-	-	+	+	-	+	+
<i>Escherichia coli invasive</i>	V	(-)	(-)	-	-	-	+	+	-	(-)	V
<i>Escherichia fergusonii</i>	+	+	+	-	-	-	+	+	V	-	V
<i>Escherichia hermannii</i>	+	-	+	-	-	-	+	+	+	-	V
<i>Escherichia vulneris</i>	+	V	-	-	-	-	+	-	+	-	+
<i>Hafnia alvei</i>	+	+	+	-	-	V	V	-	-	-	-
<i>Klebsiella oxytoca</i>	+	+	-	+	-	+	V	+	+	-	+
<i>Klebsiella ozaenae</i>	+	(-)	-	(-)	-	-	+	-	+	-	+
<i>Klebsiella pneumoniae</i>	+	+	-	+	-	+	V	-	+	-	+
<i>Klebsiella rhinoscleromatis</i>	-	-	-	-	-	-	+	-	+	-	(-)
<i>Kluyvera ascorbata</i>	+	+	+	-	-	-	+	+	-	-	V
<i>Leclercia adecarboxylata</i>	+	-	-	(-)	-	-	+	+	+	-	+
<i>Morganella morganii ssp. morganii</i>	-	-	+	+	+	-	+	+	-	-	-
<i>Morganella morganii ssp. sibonii</i>	-	(-)	V	+	+	-	V	V	-	-	-
<i>Pantoea agglomerans</i>	+	-	-	-	V	+	V	-	+	-	-
<i>Pantoea dispersa</i>	+	-	-	-	-	+	V	-	V	-	V
<i>Pasteurella gallinarum</i>	-	-	+	-	-	-	-	-	V	-	V
<i>Pasteurella multocida</i>	-	-	+	-	-	-	-	+	V	-	V
<i>Proteus mirabilis</i>	-	-	+	+	+	(-)	+	-	-	-	-
<i>Proteus penneri</i>	-	-	-	+	+	-	+	-	-	-	-
<i>Proteus vulgaris</i>	-	-	-	+	+	-	-	+	-	-	-
<i>Providencia alcalifaciens</i>	-	-	-	-	+	-	+	+	-	-	-
<i>Providencia rettgeri</i>	-	-	-	+	+	-	+	+	-	-	-
<i>Providencia rustigianni</i>	-	-	-	-	+	-	V	+	-	-	V
<i>Providencia stuartii</i>	-	-	-	V	+	-	-	+	-	-	-
<i>Rahnella aquatilis</i>	+	-	-	-	-	V	V	-	+	-	V
<i>Salmonella arizona</i>	+	+	+	-	-	-	+	-	-	-	+
<i>Salmonella choleraesuis</i>	-	+	+	-	-	-	+	-	-	V	(-)
<i>Salmonella paratyphi A</i>	-	-	+	-	-	-	-	-	-	V	-
<i>Salmonella spp.</i>	-	+	+	-	-	-	+	-	-	-	+
<i>Salmonella typhi</i>	-	+	-	-	-	-	+	-	-	-	+
<i>Serratia entomophila</i>	+	-	-	-	-	+	V	-	+	-	V
<i>Serratia ficaria</i>	+	-	-	-	-	+	V	-	+	-	-
<i>Serratia fonticola</i>	+	+	+	-	-	-	+	-	+	-	V
<i>Serratia marcescens</i>	+	+	+	-	-	+	V	-	+	-	-
<i>Serratia odorifera 1</i>	+	+	+	-	-	-	V	+	V	+	+
<i>Serratia odorifera 2</i>	+	+	-	-	-	+	V	V	+	-	+
<i>Serratia plymuthica</i>	+	-	-	-	-	(-)	+	-	+	-	V
<i>Serratia rubidaea</i>	+	V	-	-	-	+	V	-	+	-	+
<i>Shigella dysenteriae_serogroup_A</i>	(-)	-	-	-	-	-	-	V	-	-	V
<i>Shigella flexneri_serogroup_B</i>	-	-	-	-	-	-	-	V	-	-	V
<i>Shigella sonnei</i>	+	-	+	-	-	-	+	-	-	+	+
<i>Shigella spp. (not sonnei)</i>	-	-	-	-	-	-	+	V	-	-	V
<i>Yersinia enterocolitica</i>	V	-	+	+	-	-	+	V	+	-	-
<i>Yersinia kristensenii</i>	V	-	+	+	-	-	-	V	+	V	-
<i>Yersinia pseudotuberculosis</i>	V	-	-	+	-	-	+	-	+	-	-
<i>Yersinia rohdei</i>	V	-	(-)	V	-	-	-	-	+	V	V
<i>Yokenella regensburgei</i>	+	+	+	-	-	-	-	-	-	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%)

= 11-89% positive.

Xylosidase	Esculin	Sucrose	Sorbitol	Trehalose	Glucose	Cellobiose	Melibiose	Salicin	Mannose	Maltose	Raffinose	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	-	-	+	+	+	+	-	+	+	(-)	-

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 $\beta$ -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	Voges Proskauer's	2-3 drops of Baritt reagent A and 1 drop of Baritt reagent B	Detects acetoin production	Colourless / Light Yellow	Pinkish red	Colourless/ slight copper
7	Methyl red	1-2 drops of Methyl red reagent	Detects acid production	Colourless	Red	Yellowish- orange
8	Indole	1-2 drops of Kovac's red reagent	Detects deamination of tryptophan	Colourless	Pinkish Red	Colourless
9	PYR	1-2 drops of PYR reagent	Detects PYR enzyme activity	Cream	Cherry Red	Cream
10	- Glucuronidase	—	For Enzymatic hydrolysis of Glucuronidase	Colourless / Light Yellow	Bluish Green	Light Yellow
11	- Galactosidase	—	For Enzymatic hydrolysis of Galactosidase	Colourless / Light Yellow	Pink	Colourless / Light Yellow
12	-Xylosidase	—	For Enzymatic hydrolysis of Xylosidase	Colourless / Light Yellow	Purple	Colourless Light Yellow

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	Sucrose	Sucrose utilization	Pinkish Red / Red	Yellow	Red / Pink
15	Sorbitol	Sorbitol utilization	Pinkish Red / Red	Yellow	Red / Pink
16	Trehalose	Trehalose utilization	Pinkish Red / Red	Yellow	Red / Pink
17	Glucose	Glucose 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	Salicin	Salicin utilization	Pinkish Red / Red	Yellow	Red / Pink
21	Mannose	Mannose utilization	Pinkish Red / Red	Yellow	Red / Pink
22	Maltose	Maltose utilization	Pinkish Red / Red	Yellow	Red / Pink
23	Raffinose	Raffinose utilization	Pinkish Red / Red	Yellow	Red / Pink
24	Lactose	Lactose 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  $\pm$  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.

#### Disclaimer :

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