Technical Data

# Hi24™ Enterobacteriaceae Identification Kit, Modified KB016

## Intended use

KB016 is a standardized, colorimetric identification system, a combination of 24 tests for the identification of *Enterobacteriaceae* species (from clinical specimen and non clinical samples using pure isolate.

## Kit Contains :

Each kit contains sufficient material to perform 5/10/20 tests. (Kit contains sterile medium in Strip1 for ONPG, Lysine utilization, Ornithine utilization, Urease detection, Phenylalanine deamination (TDA), Voges- Proskauer's, Methyl red, Indole, PYR, β-Glucuronidase, α-Galactosidase, β-Xylosidase and Strip 2 for Esculin hydrolysis and 11 different carbohydrates utilization test - Sucrose, Sorbitol, Trehalose, Glucose, Cellobiose, Melibiose, Salicin, Mannose, Maltose, Raffinose, Lactose)

1. 5/10/20 kits of Part I.
2. 5/10/20 kits of Part II.
3. Technical product insert.
4. Result Interpretation Chart and Result Entry Datasheet.
5. Identification Index.
6. TDA reagent (R036) for Phenylalanine Deaminase test.
7. Baritt reagent A (R029) for Voges-Proskauer's test.
8. Baritt reagent B (R030) for Voges-Proskauer's test.
9. Methyl Red reagent (I007) for Methyl Red test
10. Kovac's reagent (R008) for Indole test
11. PYR Reagent (R043) for PYR test

## Material Required but not supplied :

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

## Direction

### 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 Agart (M001) or a differential medium like MacConkey Agar (M082).
- 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 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 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.

### 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±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 in well no 5,-9 should be done at the end of incubation period that is after 18 -24 hours.

## Principle

Each Hi24™ Enterobacteriaceae Identification Kit, Modified is a standardized colorimetric identification system utilizing ten conventional biochemical tests, 3 chromogenic substrate utilization test 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.

## 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**

Two Sterile white opaque strips with 12 wells each. Strip 1containing sterile media for ONPG, Lysine utilization, Ornithine utilization, Urease detection, Phenylalanine deamination (TDA), Voges- Proskauer's, Methyl red, Indole, PYR, β-Glucuronidase, α-Galactosidase, β-Xylosidase and Strip 2 for Esculin hydrolysis and 11 different carbohydrates utilization test - Sucrose, Sorbitol, Trehalose, Glucose, Cellobiose, Melibiose, Salicin, Mannose, Maltose, Raffinose, Lactose

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

**Part I :**

**ONPG Test : Well No. 1**

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

**Lysine utilization: Well No. 2**

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

**Ornithine utilization: Well No. 3**

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

**Urease Test: Well No. 4**

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

**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).
- 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.

**Methyl red Test : Well No. 7**

- Add 2-3 drops of Methyl red indicator (I007).
- Positive test is indicated by a development of red colour.
- Yellowish orange colour indicates a negative reaction.

**Indole Test : Well No. 8**

- Add 2-3 drops of Kovacs reagent (R008)
- Positive test is indicated by a development of pinkish red ring.
- No red ring indicates a negative reaction.

**PYR test : Well No. 9**

- Add 1-2 drops of PYR reagent (R043).
- Positive test is indicated by development and retension of cherry red colour.
- Development of pink, orange or yellow colour indicates a negative reaction.

**β-Glucuronidase : Well No. 10**

- Positive test is indicated by colour change to blue/ bluish green
- No colour change indicates a negative reaction.

**α-Galactosidase : Well No. 11**

- Positive test is indicated by colour change to pink
- Colourless /Light yellow indicates a negative reaction.

**β-Xylosidase : Well No. 12**

- Positive test is indicated by colour change to purple
- Colourless /Light yellow indicates a negative reaction.

**Part II**

**Esculin hydrolysis: Well No. 1**

- Cream colour changes to black colour indicates a positive reaction
- No colour change or brownish yellow indicates a negative reaction

**Carbohydrate fermentation Test : Well No. 2-12**

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

| 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 | $\beta$ - Glucuronidase | — | For Enzymatic hydrdysis of Glucuronidase | Colourless / Light Yellow | Bluish Green | Light Yellow |
| 11 | $\alpha$ - Galactosidase | — | For Enzymatic hydrdysis of Galactosidase | Colourless / Light Yellow | Pink | Colourless / Light |
| 12 | $\beta$ -Xylosidase | — | For Enzymatic hydrdysis of Xylosidase | Colourless / Light Yellow | Purple | Colourless Light |

| 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 |

## Result Entry Datasheet

| No. | Test | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ONPG | | | | | | | | | | |
| 2 | Lysine utilization | | | | | | | | | | |
| 3 | Ornithine utilization | | | | | | | | | | |
| 4 | Urease | | | | | | | | | | |
| 5 | Phenylalanine Deamination | | | | | | | | | | |
| 6 | Voges Proskauer's | | | | | | | | | | |
| 7 | Methyl red | | | | | | | | | | |
| 8 | Indole | | | | | | | | | | |
| 9 | PYR | | | | | | | | | | |
| 10 | $\beta$ - Glucuronidase | | | | | | | | | | |
| 11 | $\alpha$ - Galactosidase | | | | | | | | | | |
| 12 | $\beta$ -Xylosidase | | | | | | | | | | |
| 13 | Esculin hydrolysis | | | | | | | | | | |
| 14 | Sucrose | | | | | | | | | | |
| 15 | Sorbitol | | | | | | | | | | |
| 16 | Trehalose | | | | | | | | | | |
| 17 | Glucose | | | | | | | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | Cellobiose | | | | | | | | | | |
| 19 | Melibiose | | | | | | | | | | |
| 20 | Salicin | | | | | | | | | | |
| 21 | Mannose | | | | | | | | | | |
| 22 | Maltose | | | | | | | | | | |
| 23 | Raffinose | | | | | | | | | | |
| 24 | Lactose | | | | | | | | | | |

## 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.

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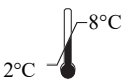
IVD ***In vitro* diagnostic medical device**




**Storage temperature**

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

**CE Marking**

**Do not use if package is damaged**

**Do not re-use**

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