Technical Data

# KB001 HiIMViC™ Biochemical Test Kit

KB001 is a combination of 12 tests for differentitation of Enterobacteriaceae species. Kit contains sterile media for Indole, Methyl red, Voges Proskauer's, Citrate utilization tests and 8 different carbohydrates-Glucose, Adonitol, Arabinose, Lactose, Sorbitol, Mannitol, Rhamnose, Sucrose. Reagents supplied with kit : Kovac's Reagent (R008) for Indole Test Methyl Red Indicator (I007), Barritt Reagent A (R029) and Barritt Reagent B (R030) for VP Test

## Principle

Each HiIMViC™ kit is a standardized colorimetric identification system utilizing four conventional biochemical tests and eight 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 as a colour change in the media that can be either interpreted visually or after addition of the reagent.

## Kit contents

1. Each kit contains sufficient material to perform 10 tests.
2. 10 kits of KB001.
3. Technical product insert.
4. Result Interpretation Chart and Result Entry Datasheet.
5. Identification Index.
6. Kovac's reagent (R008) for Indole test.
7. Methyl Red reagent (I007) for Methyl Red test.
8. Baritt reagent A (R029) for Voges-Proskauer's test.
9. Baritt reagent B (R030) for Voges-Proskauer's test.

## Instructions for use

1. **Preparation of inoculum**
   - KB001 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 Brain Heart Infusion Agar (M211). Pick up a single well isolated colony and inoculate in 5ml 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. Alternatively, a homogeneous suspension made in 2-3 ml sterile saline can be used for inoculation. The density of the suspension should be adjusted to 0.1 OD at 620nm or 0.5 Mcfarland standard.

Note:
- Erroneous false negative results may be obtained if the inoculum turbidity is less than 0.1 OD.
- Results are more prominent when enriched culture instead of suspension.

2. **Inoculation of the kit**
   - Open the kit aseptically. Peel off the sealing tape.
   - Inoculate each well with 50 µl of the above inoculum by surface inoculation method.
   - Alternatively the kit can be inoculated by stabbing each individual well with a loopful of inoculum.

3 **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 Result Interpretation Chart. Addition of reagents in well nos 1,2, and 3 should be done at the end of incubation period, that is after 18 - 24 hours. Following reagents to be added to the respective wells.

## Indole Test : Well No. 1

- Add 1-2 drops of Kovac's reagent (R008). Development of reddish pink colour within 10 seconds indicates positive reaction.
- Reagent remains pale coloured if the test is negative.

## Methyl Red Test : Well No. 2

- 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.

## Voges Proskaeur's Test : Well No. 3

- Add 1-2 drops of Baritt reagent A (R029) and 1-2 drops of Baritt reagent B(R030).
- Pinkish red colour development within 5-10 minutes indicates a positive test.
- No change in colour or a slight copper colour (due to reaction of Baritt reagent A with Baritt reagent B) denotes a negative reaction.

## Identification Index of various *Enterobacteriaceae species*

| Tests | Indole | Methyl red | Voges Proskauer's | Citrate Utilization | Glucose | Adonitol | Arabinose | Lactose | Sorbitol | Mannitol | Rhamnose | Sucrose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Budvicia aquatica* | – | + | – | – | + | – | V | V | – | V | + | – |
| *Buttiauxella agrestis* | – | + | – | + | + | – | + | + | – | + | + | – |
| *Cedecea davisae* | – | + | V | + | + | – | – | V | – | + | – | + |
| *Cedecea lapagei* | - | V | V | + | + | – | – | V | – | + | – | – |
| *Cedecea neteri* | – | + | V | + | + | – | – | V | + | + | - | + |
| *Citrobacter amalonaticus* | + | + | – | V | + | – | + | V | + | + | + | V |
| *Citrobacter diversus* | + | + | – | + | + | + | + | V | + | + | + | V |
| *Citrobacter freundii* | – | + | – | + | + | – | + | V | + | + | + | V |
| *Enterobacter aerogenes* | – | – | + | + | + | + | + | + | + | + | + | + |
| *Enterobacter amnigenus (Biogroup I)* | – | – | + | V | + | – | + | V | – | + | + | + |
| *Enterobacter amnigenus (Biogroup II)* | – | V | + | + | + | – | + | V | + | + | + | – |
| *Enterobacter taylorae (E. cancerogenus)* | – | – | + | + | + | – | + | – | – | + | + | – |
| *Enterobacter cloacae* | – | – | + | + | + | – | + | + | + | + | + | + |
| *Enterobacter gergoviae* | – | – | + | + | + | – | + | V | – | + | + | + |
| *Enterobacter sakazakii* | V | – | + | + | + | – | + | + | – | + | + | + |
| *Escherichia coli* | + | + | – | – | + | – | + | + | + | + | V | V |
| *Escherichia coli, inactive* | V | + | – | – | + | – | V | V | V | + | V | V |
| *Escherichia blattae* | – | + | – | V | + | – | + | – | – | – | + | – |
| *Escherichia fergusonii* | + | + | – | V | + | + | + | – | – | + | + | – |
| *Escherichia hermanii* | + | + | – | – | + | – | + | V | – | + | + | V |
| *Escherichia vulneris* | – | + | – | – | + | – | + | V | – | + | + | – |
| *Ewingella americana* | – | V | + | + | + | – | – | V | – | + | V | – |
| *Hafnia alvei* | – | V | V | – | + | – | + | – | – | + | + | – |
| *Klebsiella oxytoca* | + | V | + | + | + | + | + | + | + | + | + | + |
| *Klebsiella pneumoniae subspecies ozaenae* | – | + | – | V | + | + | + | V | V | + | V | V |
| *Klebsiella pneumoniae subspecies pneumoniae* | – | V | + | + | + | + | + | + | + | + | + | + |
| *Klebsiella pneumoniae subspecies rhinoscleromatis* | – | + | – | – | + | + | + | – | + | + | + | V |
| *Klebsiella terrigena* | – | V | + | V | + | + | + | + | + | + | + | + |
| *Kluyvera ascorbata* | + | + | – | + | + | – | + | + | V | + | + | + |
| *Leclercia adecarboxylata (Escherichia adecarboxylata)* | + | + | – | – | + | + | + | + | – | + | + | V |
| *Morganella morganii* | + | + | – | – | + | – | – | – | – | – | – | – |
| *Pantoea agglomerans* | – | V | + | + | + | – | + | V | – | + | + | + |
| *Pantoea dispersa* | – | V | + | + | + | – | V | – | – | + | + | + |
| *Proteus mirabilis* | – | + | V | V | + | – | – | – | – | – | – | V |
| *Proteus myxofaciens* | – | + | + | + | + | – | – | – | – | – | – | + |
| *Proteus penneri* | – | + | – | – | + | – | – | – | – | – | – | + |
| *Proteus vulgaris* | + | + | – | 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.

## Identification Index of various *Enterobacteriaceae species*

| Tests | Indole | Methyl red | Voges Proskauer's | Citrate Utilization | Glucose | Adonitol | Arabinose | Lactose | Sorbitol | Mannitol | Rhamnose | Sucrose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Providencia alcalifaciens* | + | + | – | + | + | + | – | – | – | – | – | V |
| *Providencia rettgeri* | + | + | – | + | + | + | – | – | – | + | V | V |
| *Providencia rustigianii* | + | V | – | V | + | – | – | – | – | – | – | V |
| *Rahnella aquatilis* | – | V | + | + | + | – | + | + | + | + | + | + |
| *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* | – | V | + | + | + | – | – | – | – | + | – | + |
| *Serratia ficaria* | – | V | V | + | + | – | + | V | + | + | V | + |
| *Serratia fonticola* | – | + | – | + | + | + | + | + | + | + | V | V |
| *Serratia marcescens* | – | V | + | + | + | V | – | – | + | + | – | + |
| *Serratia odorifera (Biogroup I)* | V | + | V | + | + | V | + | V | + | + | + | + |
| *Serratia odorifera (Biogroup II)* | V | V | + | + | + | V | + | + | + | + | + | – |
| *Serratia plymuthica* | – | + | V | V | + | – | + | V | V | + | – | + |
| *Serratia proteamaculans* | – | V | V | + | + | – | + | – | V | + | V | + |
| *Serratia rubidaea* | – | V | + | + | + | + | + | + | – | + | – | + |
| *Shigella boydii, Shigella flexneri, Shigella dysenteriae* | V | + | – | – | + | – | V | – | V | + | – | – |
| *Shigella sonnei* | – | + | – | – | + | – | + | – | – | + | V | – |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Yersinia enterocolitica* | V | + | – | – | + | – | + | – | + | + | – | + |
| *Yersinia frederiksenii* | + | + | – | V | + | – | + | V | + | + | + | + |
| *Yersinia intermedia* | + | + | – | – | + | – | + | V | + | + | + | + |
| *Yersinia pestis* | – | V | – | – | + | – | + | – | V | + | – | – |
| *Yersinia pseudotuberculosis* | – | + | – | – | + | – | V | – | – | + | V | – |

Note : Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.

+ = Positive (more than 90%)
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## Result Interpretation chart

| No. | Test | Reagents to be added after incubation | Principle | Original colour of the medium | Positive reaction | Negative reaction |
|---|---|---|---|---|---|---|
| 1 | Indole | 1-2 drops of Kovac's red reagent | Detects deamination of tryptophan | Colourless | Reddish pink | Colourless |
| 2 | Methyl red | 1-2 drops of Methyl red reagent | Detects acid production | Colourless/ light yellow | Red | Yellowish - orange |
| 3 | 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 |
| 4 | Citrate utilization | — | Detects capability of organism to utilize citrate as a sole carbon source | **Green** | **Blue** | **Green** |
| 5 | Glucose | — | Carbohydrate utilization | Pinkish Red / Red | Yellow | Red |
| 6 | Adonitol | — | Carbohydrate utilization | Pinkish Red / Red | Yellow | Red |
| 7 | Arabinose | — | Carbohydrate utilization | Pinkish Red / Red | Yellow | Red |
| 8 | Lactose | — | Carbohydrate utilization | Pinkish Red / Red | Yellow | Red |
| 9 | Sorbitol | — | Carbohydrate utilization | Pinkish Red / Red | Yellow | Red |
| 10 | Mannitol | — | Carbohydrate utilization | Pinkish Red / Red | Yellow | Red |
| 11 | Rhamnose | — | Carbohydrate utilization | Pinkish Red / Red | Yellow | Red |
| 12 | Sucrose | — | Carbohydrate utilization | Pinkish Red / Red | Yellow | Red |

## 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 upto 48 hours. Orange colour after 48 hours of incubation should be interpreted as a negative reaction.
3. In case of Lysine and Ornithine decarboxylation reaction, incubation upto 48 hours may be required.
4. At times organisms give contradictory 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 obtained 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 disposable bag.

## Storage and Shelf-life

On receipt store at 2-8$^{0}$C. Shelf-life is 12 months.

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