# KB002 HiAssorted™ Biochemical Test Kit (for Gram-negative rods)

## Introduction

KB002 is a test system that can be used for identification of gram-negative rods. HiAssorted™ Biochemical Test 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 HiAssorted™ Biochemical Test kit is a standardized colorimetric identification system utilizing seven conventional biochemical tests and five 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 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 KB002
3. Technical product insert.
4. Result Interpretation Chart and Result Entry Datasheet.
5. Identification Index.
6. TDA reagent (R036) for Phenylalanine Deaminase test.
7. Sulphanilic acid 0.8% (R015) for Nitrate Reduction test.
8. N, N-Dimethyl-1-Napthylamine Reagent (R009) for Nitrate Reduction test.

## Storage and Shelf-life

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

## Instructions for use

1. **Preparation of inoculum**
   - KB002 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) or a differential medium like MacConkey Agar (M082). 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 ≥ 0.1OD 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 0.1OD 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 is used 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 also 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 no 5 and well no 6 should be done at the end of incubation period that is after 18 - 24 hours. Following reagents are to be added to the respective wells.

**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.

## 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 |

## 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.

## 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 |

### 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

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

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