Instructions for use

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.

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.

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 comon 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. Incubation 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. Interpretation of results:

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

Part I: Phenylalanine Deaminase Test: Well No. 5
   - Add 2-3 drops of TDA reagent (R036) for Phenylalanine Deaminase test.

Voges Proskauer’s Test: Well No. 6
   - Add 1-2 drops of Barritt reagent A (R029) for Voges-Proskauer’s test.
   - Add 1-2 drops of Barritt reagent B (R030).

Methyl Red Test: Well No. 7
   - Add 1-2 drops of Methyl Red reagent (I007).

Indole Test: Well No. 8
   - Add 1-2 drops of Kovac’s reagent (R008) for Indole test.
   - Add 1-2 drops of PYR Reagent (R043).

PYR Test: Well No. 9
   - Positive test is indicated by development and reaction of cherry red colour.
   - Development of Pink, Orange or Yellow colour indicates a negative reaction.
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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%)

11-88% positive.
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Please refer disclaimer Overleaf.
### Strip I

<table>
<thead>
<tr>
<th>No.</th>
<th>Test</th>
<th>Reagents to be added after incubation</th>
<th>Principle</th>
<th>Original colour of the medium</th>
<th>Positive reaction</th>
<th>Negative reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ONPG</td>
<td>—</td>
<td>Detects β-galactosidase activity</td>
<td>Colourless</td>
<td>Yellow</td>
<td>Colourless</td>
</tr>
<tr>
<td>2</td>
<td>Lysine utilization</td>
<td>—</td>
<td>Detects lysine decarboxylation</td>
<td>Light Purple</td>
<td>Purple / Dark Purple</td>
<td>Purple / Yellow</td>
</tr>
<tr>
<td>3</td>
<td>Ornithine utilization</td>
<td>—</td>
<td>Detects ornithine decarboxylation</td>
<td>Light Purple</td>
<td>Purple / Dark Purple</td>
<td>Purple / Yellow</td>
</tr>
<tr>
<td>4</td>
<td>Urease</td>
<td>—</td>
<td>Detects urase activity</td>
<td>Orangish yellow</td>
<td>Pink</td>
<td>Orangish yellow</td>
</tr>
<tr>
<td>5</td>
<td>Phenylalanine</td>
<td>2-3 drops of TDA reagent</td>
<td>Detects phenylalanine decarboxylation</td>
<td>Colourless</td>
<td>Yellow</td>
<td>Colourless</td>
</tr>
<tr>
<td>6</td>
<td>Voges Proskauer's</td>
<td>2-3 drops of Barritt reagent A and B</td>
<td>Detects acid production</td>
<td>Colourless</td>
<td>Light Red</td>
<td>Colourless / Slight copper</td>
</tr>
<tr>
<td>7</td>
<td>Methyl red</td>
<td>1-2 drops of Methyl red reagent</td>
<td>Detects acid production</td>
<td>Colourless</td>
<td>Red / Black</td>
<td>Colourless / Orange</td>
</tr>
<tr>
<td>8</td>
<td>Indole</td>
<td>1-2 drops of Kovac's red reagent</td>
<td>Detects tryptophan</td>
<td>Colourless</td>
<td>Black / Red</td>
<td>Colourless</td>
</tr>
<tr>
<td>9</td>
<td>PYR</td>
<td>1-2 drops of PYR reagent</td>
<td>Detects PR enzymes activity</td>
<td>Colourless</td>
<td>Black / Red</td>
<td>Colourless</td>
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<tr>
<td>10</td>
<td>β-Glucoronidase</td>
<td>—</td>
<td>For Enzymatic hydrolysis of Glucuronidase</td>
<td>Yellow / Light Yellow</td>
<td>Red / Pink</td>
<td>Yellow / Light Yellow</td>
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<tr>
<td>11</td>
<td>α-Glucoronidase</td>
<td>—</td>
<td>For Enzymatic hydrolysis of Glucuronidase</td>
<td>Yellow / Light Yellow</td>
<td>Pink</td>
<td>Colourless / Light Yellow</td>
</tr>
<tr>
<td>12</td>
<td>β-Xylosidase</td>
<td>—</td>
<td>For Enzymatic hydrolysis of Xylosidase</td>
<td>Yellow / Light Yellow</td>
<td>Purple</td>
<td>Colourless Light Yellow</td>
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</tbody>
</table>

### Strip II

<table>
<thead>
<tr>
<th>No.</th>
<th>Test</th>
<th>Principle</th>
<th>Original colour of the medium</th>
<th>Positive reaction</th>
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<td>Trehalose</td>
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<td>Cream</td>
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<td>17</td>
<td>Glucose</td>
<td>Glucose utilization</td>
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<td>Black</td>
<td>Cream</td>
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<td>18</td>
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<td>Black</td>
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<td>19</td>
<td>Melibiose</td>
<td>Melibiose utilization</td>
<td>Cream</td>
<td>Black</td>
<td>Cream</td>
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<td>20</td>
<td>Salicine</td>
<td>Salicine utilization</td>
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<td>Black</td>
<td>Cream</td>
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<td>21</td>
<td>Mannose</td>
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<td>Black</td>
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<td>22</td>
<td>Maltose</td>
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<td>Black</td>
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<td>Raffinose</td>
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<td>Black</td>
<td>Cream</td>
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<tr>
<td>24</td>
<td>Lactose</td>
<td>Lactose utilization</td>
<td>Cream</td>
<td>Black</td>
<td>Cream</td>
</tr>
</tbody>
</table>

### 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.

### Disclaimer:

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