

KB010 HiE. Coli™ Identification Kit

Introduction

KB010 is a comprehensive test system that can be used for identification and differentiation of *Escherichia coli*. *Escherichia coli* are gram negative, lactose fermenting coccobacillary rods which are frequently isolated from food, feces, water and other relevant clinical samples. HiE.Coli identification kit can be used for screening pathogenic organisms and 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 KB010 kit is a standardized colorimetric identification system utilizing eight conventional biochemical tests and four carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation *E. coli* exhibit metabolic changes 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.
2. 10 kits of KB010.
3. Technical product insert.
4. Result Interpretation Chart and Result Entry Datasheet.
5. Identification Index.
6. Baritt reagent A (R029) for Voges-Proskauer's test.
7. Baritt reagent B (R030) for Voges-Proskauer's test.
8. Methyl Red reagent (I007) for Methyl Red test.
9. Kovac's reagent (R008) for Indole test
10. Sulphanilic acid 0.8%(R015) for Nitrate test
11. N,N-Dimethyl-1-Naphthylamine reagent(R009) for Nitrate test

Instructions for use

1. Preparation of inoculum

- KB010 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 (M210). Incubate at 35-37°C for 4-6 hours till the density of the inoculum is ≥ 0.1 OD at 620nm or 0.5 Mcfarland standard.
- Alternatively, a homogenous suspension adjusted to 0.1 OD at 620nm or 0.5 Mcfarland standard can also be used for inoculation.

Note :

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

2. Inoculation of the kit :

- Open the kit aseptically. Peel off the sealing foil.
- Inoculate each well with 50 μ l 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° \pm 2°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#1, 2, and 4 should be done at the end of incubation period that is after 18 - 24 hours.

Following reagents to be added to the respective wells

Methyl Red Test : Well No. 1

- 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 Proskauer's Test : Well No. 2

- Add 2-3 drops of Baritt reagent A (R029) and 1-2 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.

Indole Test : Well No. 4

- 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

Identification Index of various Escherichia species

| Tests | Methyl Red | Voges Proskauer's | Citrate utilization | Indole | Glucuronidase | Nitrate | ONPG | Lysine | Lactose | Glucose | Sucrose | Sorbitol |
|--------------------------|------------|-------------------|---------------------|--------|---------------|---------|------|--------|---------|---------|---------|----------|
| <i>E. coli</i> | + | - | - | + | + | + | + | + | + | + | V | + |
| <i>E. coli, inactive</i> | + | - | - | V | - | + | V | V | V | + | V | V |
| <i>E. fergusonii</i> | + | - | V | + | - | + | V | + | - | + | - | - |
| <i>E. hermannii</i> | + | - | - | + | - | + | + | - | V | + | V | - |
| <i>E. vulneris</i> | + | - | - | - | - | + | + | V | V | + | - | - |
| <i>E. blattae</i> | + | - | 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 = Variable (11-89%)

Result Interpretation chart

| No. | Test | Reagents to be added after incubation | Principle | Original colour of the medium | Positive reaction | Negative reaction |
|-----|---------------------|---|---|-------------------------------|-----------------------|---------------------------|
| 1 | Methyl red | 1-2 drops of Methyl red reagent | Detects acid production | Colourless | Red | Yellowish-orange |
| 2 | 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 |
| 3 | Citrate utilization | — | Detects capability of organism to utilize citrate as a sole carbon source | Green | Blue | Green |
| 4 | Indole | 1-2 drops of Kovac's red reagent | Detects deamination of tryptophan | Colourless | Reddish pink | Colourless |
| 5. | Glucuronidase | — | Detects Glucuronidase activity | Colourless | Bluish green | Colourless |
| 6. | Nitrate reduction | 1-2 drops of sulphanilic acid and 1-2drops of N,N Dimethyl-1- Naphthylamine | Detects Nitrate reduction | Colourless | Pinkish red | Colourless |
| 7 | ONPG | — | Detects β -galactosidase activity | Colourless | Yellow | Colourless |
| 8 | Lysine utilization | — | Detects Lysine decarboxylation | Olive green to | Purple / Light purple | Yellow Dark purple |
| 9 | Lactose | — | Lactose utilization | Pinkish Red / Red | Yellow | Red / Pink |
| 10 | Glucose | — | Glucose utilization | Pinkish Red / Red | Yellow | Red / Pink |
| 11 | Sucrose | — | Sucrose 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 \pm 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.

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 and Shelf-life

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

**Disclaimer :**

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