Technical Data

KBM001 HiMotility™ Biochemical kit for *Escherichia coli*

Introduction

KBM001 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 KBM001 kit is a standardized colorimetric identification system utilizing seven conventional biochemical tests including motility 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 which are indicated by a spontaneous color change in the media that can be either interpreted visually or after addition of reagent wherever required.

Kit contents

1. Each kit contains sufficient material to perform 10 tests.
2. 10 kits of KBM001.
3. Technical product insert.
4. Result Interpretation Chart and Result Entry Datasheet.
5. Kovac's reagent (R008) for Indole test.
6. Sulphanilic acid 0.8% (R015) for Nitrate Reduction test.
7. N, N-Dimethyl-1-Napthylamine Reagent (R009) for Nitrate Reduction test.

Instructions for use

1. **Preparation of inoculum**
   - KBM001 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 instead of suspension.

2. **Inoculation of the kit :**
   - Open the kit aseptically. Peel off the sealing foil.
   - Stab inoculate the 1st well. DO NOT INOCULATE THE 2ND WELL.
   - Inoculate the remaining kit (well no.3-12) by stabbing each individual well (except well no. 2) with a loopful of inoculum. Inoculum should reach the bottom of the wells.

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.

Motility : Well No. 1

- Motility is seen as movement of bluish green growth from 1st well to 2nd well.

Indole Test : Well No. 3

- 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

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.
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Identification Index of various *Escherichia* species

Tests	Motility	Motility	Indole	Citrate utilization	Glucuronidase	Nitrate	ONPG	Lysine	Lactose	Glucose	Sucrose	Sorbitol
E. coli	+		+	-	+	+	+	+	+	+	V	+
E. coli, inactive	-		V	-	-	+	V	V	V	+	V	V
E. fergusonii	+		+	V	-	+	V	+	-	+	-	-
E. hermannii	+		+	-	-	+	+	-	V	+	V	-
E. vulneris	+		-	-	-	+	+	V	V	+	-	-
E. blattae	-		-	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	Motility	—	—	Colourless	Bluish green growth seen	No growth seen
2	Motility	—	Detects motility	Colourless	Movement of Bluish green growth from 1st well to 2nd well	No growth seen
3	Indole	1-2 drops of Kovac's indole reagent	Detects deamination of tryptophan	Colourless	Reddish pink	Colourless
4	Citrate utilization	—	Detects capability of organism to utilize citrate as a sole carbon source	Green	Blue	Green
5.	Glucorinidase	—	Detects Glucorinidase activity	Colourless	Bluish green	Colourless
6.	Nitrate reduction	1-2 drops of sulphanilic acid and 1-2drops of N,N Dimethyl-1- Napthylamine	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 Light Purple	Purple / Dark Purple	Yellow
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. In case of Carbohydrate fermentation test some microorganisms may 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.
2. In case of Lysine utilization, incubation up to 48 hours may be required.
3. At times organisms give contradictory 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 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.

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 between 2-8 °C. Shelf-life is 12 months.

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