Technical Data

KBM003A HiMotility™ Biochemical Kit for Listeria

Introduction

Listeria species are ubiquitous organisms and are the most frequent contaminants of various kinds of food products. Human Listeriosis occurs in sporadic and epidemic forms and has 20% to 30% fatality rate. The pathogenic strain of humans, *L. monocytogenes* primarily causes Meningitis, Encephalitis, Septicemia, and in pregnant women it may cause abortion, still birth or premature birth. KBM003A can be used for screening food samples 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 kit is given in the identification index provided with the kit.

Principle

Each KBM003A is a standardized, colorimetric test system based on motility, carbohydrate utilization and other biochemical tests specific for the identification of *Listeria* species. The tests are based on the principle of pH change and substrate utilization. *Listeria* species on incubation exhibit metabolic changes which are indicated by a colour 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 KBM003A.
3. Technical product insert
4. Result Interpretation Chart and Result Entry Datasheet
5. Identification Index
6. Sulphanilic acid 0.8% (R015)
7. α-Naphthylamine Solution (R009)
8. Baritt reagent A (R029)
9. Baritt reagent B (R030)
10. Methyl Red reagent (I007)

Instructions for use

Preparation of inoculum

- KBM003A cannot be used directly on clinical or food samples. The organism to be identified has to be first isolated and purified.
- Isolate the organism to be identified on Listeria Identification Agar Base (PALCAM) (M1064) .
- Pick up a single isolated colony and inoculate in 5 ml Brain Heart Infusion Broth (M210) and incubate at 35-37^{0}C for 6 to 8 hours until inoculum turbidity is 0.5 to 1.0 McFarland standard.
- 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 0.5 to 1.0 McFarland standard.
- Note : Erroneous false negative result may be obtained if the inoculum turbidity is less than 0.5 McFarland standard.
- Results are more prominent if an enriched culture is used instead of suspension.

Method of Inoculation :

- 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.

Interpretation of results

- Note the results in Result Entry Datasheet.
- Interpret results as per the standards given in the Identification index.
- Addition of reagents in well no 4, 5 and 6 should be done at the end of incubation period that is after 24 to 48 hours.

Motility + Esculin Hydrolysis : Well No.1 and 2

- Motility:Motility is observed as movement of growth from 1st well to 2nd well accompanied with blackening of the medium.
- Esculin Hydrolysis :Esculin Hydrolysis is seen as blackening in the 1st well and wherever the motile organisms have moved in the 2nd well.

Catalase : Well No. 3

- Scrape a loopful of growth from the surface of the 3rd well. Dip the loop in a small clean test tube with 3% H_2O_2.
- Positive catalase test is seen as effervescence coming out from the surface of the loop. No effervescence is observed in case of negative catalase test.

Note : 3% H_2O_2 solution has to be freshly prepared.

Nitrate Reduction : Well No. 4

- Add 1-2 drops of Sulphanilic acid (R015) and α-Naphthylamine Solution (R009).
- Immediate development of pinkish red colour on addition of reagent indicates positive reaction.
- No change in colour indicates negative reaction.

Voges-Proskauer's Test : Well No. 5

- Add 2-3 drops of Barritt reagent A (R029) and 1-2 drops of Barritt reagent B (R030) .
- On addition of reagent pinkish red colour is observed within 10 minutes.
- No change in colour or a slight copper colour (due to reaction of Baritt reagent A and Baritt reagent B) denotes a negative reaction.

Methyl red Test : Well No. 6

- Add 1-2 drops of Methyl Red Indicator (I007).
- Reagent remains distinct red if the test is positive.
- Reagent decolourises and becomes yellow if the test is negative.

Carbohydarte Fermentation Test : Well No. 7 to Well No 12

- Colour of the medium changes from red to yellow due to acid production if the test is positive.
- Medium remains red in colour if the test is negative.

Identification Index of various Listeria species

Tests	*Motility + Esculin Hydrolysis*	*Catalase*	*Nitrate Reduction*	*Voges Proskauer's*	*Methyl red*	*Xylose*	*Lactose*	*-Methyl-D-Mannoside*	*Rhamnose*	*Glucose (Dextrose)*	*Mannitol*
Listeria grayi	+	+	-	+	+	-	+	+	v	+	+
Listeria monocytogenes	+	+	-	+	+	-	v	+	+	+	-
Listeria innocua	+	+	-	+	+	-	+	+	+	+	-
Listeria seeligeri	+	+	NR	+	+	+	NR	-	-	+	-
Listeria ivannovii Sub sp. ivannovii	+	+	-	+	+	+	-	-	-	+	-
Listeria ivannovii Sub sp. londoniesis	+	+	-	+	+	+	-	-	-	+	-
Listeria welshimeri	+	+	NR	+	+	+	NR	-	+	+	-

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%) NR = Not Reported v = Variable reaction

Result interpretation chart

Well No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1+2	Motility + Esculin Hydrolysis	—	Detects motility and Esculin hydrolysis	Cream	Movement of growth in the second well with simultaneous blackening	No change in the second well
3	Catalase	3% H_2O_2 solution	Detects Catalase activity	Colourless	Effervescence coming out from the loop	No Effervescence seen
4.	Nitrate Reduction	1-2 drops ofsulphanilic acid and 1-2 drops of α-Naphthylamine Solution	Detects Nitrate reduction	Colourless	Pinkish Red	Colourless
5	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
6	Methyl red	1-2 drops of Methyl red reagent	Detects acid production	Colourless	Red	Yellowish - orange
7	Xylose	—	Xylose utilization	Pinkish Red / Red	Yellow	Red / Pink
8	Lactose	—	Lactose utilization	Pinkish Red / Red	Yellow	Red / Pink
9	-Methyl-D mannoside	—	-Methyl-D mannoside utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Rhamnose	—	Rhamnose utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Glucose (Dextrose)	—	Glucose (Dextrose) utilization	Pinkish Red / Red	Yellow	Red / Pink
12	Mannitol	—	Mannitol utilization	Pinkish Red / Red	Yellow	Red / Pink

Please refer disclaimer Overleaf.

Important points to be taken into consideration while interpreting the result

1. In case of Carbohydrate fermentation test some microorganisms show weak reaction. In this case record the reaction as ± and incubate further for 24 hours. Orange colour after 72 hours of incubation should be interpreted as a negative reaction.
2. At times organisms give contradictory result because of mutation or the media used for isolation, cultivation and maintenance.
3. 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. 3% H_2O_2 is a extremely caustic solution, so avoid contact with skin. In case it does get on the skin, immediately flood the area with70% Ethanol and not water, to neutralize the action.

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

Kits/LQ :	Store at 2-8°C,	Shelf life - 12 months
Reagents :	Store at 10-30°C	Shelf life -18 months

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