

KB012A

HiListeria™ Identification Kit

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. KB012A 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 KB012A 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 KB012A
3. Technical product insert
4. Result Interpretation Chart and Result Entry Datasheet
5. Identification Index
6. Sulphanilic acid 0.8%(R015)
7. N,N-Dimethyl-1-Naphthylamine reagent(R009)
8. Baritt reagent A(R029)
9. Baritt reagent B(R030)
10. Methyl Red reagent(I007)

Instructions for use

Preparation of inoculum

- KB012A 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 either PALCAM Agar (M1064) or Tryptose Agar (M538) with or without blood. Pick up a single isolated colony and inoculate in 5 ml Brain Heart Infusion Broth (M210) and incubate at 35-37°C for 6 to 8 hours until inoculum turbidity is 1.0 OD at 620nm.

Note : Erroneous false negative result may be obtained if the inoculum turbidity is less than 1.0 OD.

Method of Inoculation :

- Open the kit aseptically. Peel off the sealing foil.
- Inoculate each well with 50µl of the above inoculum by surface inoculation method
- Alternately, the kit can also be inoculated by stabbing each individual well with a loopful of inoculum.

Incubation : Temp. of Incubation : 35-37°C. Duration of Incubation : 24-48 hrs.

Identification Index of various *Listeria* species

Tests	Catalase	Nitrate Reduction	Esculin Hydrolysis	Voges Proskauer's	Methyl red	Xylose	Lactose	Glucose	-Methyl-D-Mannoside	Rhamnose	Sucrose	Mannitol
<i>Listeria grayi</i>	+	-	+	+	+	-	+	+	+	v	-	+
<i>Listeria monocytogenes</i>	+	-	+	+	+	-	v	+	+	+	v	-
<i>Listeria innocua</i>	+	-	+	+	+	-	+	+	+	+	v	-
<i>Listeria seeligeri</i>	+	NR	+	+	+	+	NR	+	-	-	v	-
<i>Listeria ivannovii Sub sp. ivannovii</i>	+	-	+	+	+	+	-	+	-	-	v	-
<i>Listeria ivannovii Sub sp. londoniensis</i>	+	-	+	+	+	+	-	+	-	-	v	-
<i>Listeria welshimeri</i>	+	NR	+	+	+	+	NR	+	-	+	+	-

Note : Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.

+ = Positive (more than 90 %) - = Negative NR = Not Reported v = Variable reaction

Result interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Catalase	3% H ₂ O ₂ solution	Detects Catalase activity	Colourless	Effervescence coming out from the loop	No Effervescence seen
2	Nitrate Reduction	1-2 drops of sulphanic acid and 1-2 drops of N, N-Dimethyl-1-Naphylamine	Detects Nitrate reduction	Colourless	Pinkish Red	Colourless
3	Esculin Hydrolysis	—	Detects Esculin hydrolysis	Cream	Black	Cream
4	Voges Proskauer's	1-2 drops of Barritt reagent A and 1-2 drops of Barritt reagent B	Detects acetoin production	Colourless/ Light yellow	Pinkish red	Colourless/ slight copper
5	Methyl red	1-2 drops of Methyl red reagent	Detects acid production	Colourless	Red	Yellowish-orange
6	Xylose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
7	Lactose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
8	Glucose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
9	α-Methyl-D mannocide	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Rhamnose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Sucrose	—	Carbohydrate utilization	Orangish Red	Yellow	Orangish Red
12	Mannitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink

Interpretation of results

- Interpret results as per the standards given in the Result Interpretation Chart. Addition of reagents in well no 2, 4, 5, should be done at the end of incubation period that is after 24 to 48 hours.

Catalase Test : Well No. 1

- Scrape a loopful of growth from the surface of the 3rd well. Dip the loop in a small clean test tube with 3% H₂O₂.
- 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% H₂O₂ solution has to be freshly prepared.

Nitrate Reduction : Well No. 2

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

Esculin Hydrolysis : Well No.3

- Positive reaction is indicated by blackening in the 3rd well.

Voges-Proskauer's Test : Well No. 4

- Add 3-4 drops of Barritt reagent A (5% α-Naphthol in absolute ethanol, R029) and 1 - 2 drops of Barritt reagent B (40% Potassium hydroxide, 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 Barritt reagent A and Barritt reagent B) denotes a negative reaction.

Methyl red Test : Well No. 5

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

Carbohydrate Fermentation Test : Well No. 6 to Well No 12

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

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 for 24 hours. Orange colour after 72 hours of incubation should be interpreted as a negative reaction.
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. Reagents should not come in contact with skin, eyes or clothing. 3% H₂O₂ is a extremely caustic solution, so avoid contact with skin. In case it does get on the skin, immediately flood the area with 70% 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

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

**Disclaimer :**

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.