Technical Data

KB011 HiSalmonella™ Identification kit

Introduction

KB011 is a comprehensive test system that can be used for identification of gram-negative *Salmonella* species. HiSalmonella™ identification kit can be used for screening pathogenic organisms from feces, urine, blood and other relevant clinical specimen. It 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 KB011 kit is a standardized colorimetric identification system utilizing seven conventional biochemical tests and five carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation *Salmonella* exhibit metabolic changes which are indicated by a color change in the media that can be either interpreted visually or after addition of the reagent.

Kit contents

1. Each kit contains sufficient material to perform 10 tests.
2. 10 kits of KB011.
3. Technical product insert.
4. Result Interpretation Chart and Result Entry Datasheet.
5. Baritt reagent A (R029) for Voges-Proskauer's test
6. Baritt reagent B (R030) for Voges-Proskauer's test
7. Methyl red reagent (I007) for MR test

Instructions for use

1. **Preparation of inoculum**
   - KB011 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 Brain Heart Infusion Agar (M211). 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 tape.
   - 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 **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. Addition of reagents in well#1 and 2 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 Proskaeur's Test : Well No. 2

- Add 2-3 drops of Baritt reagent A (R029) and 1 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 copper colour (due to reaction of Baritt reagent A with Baritt reagent B) denotes a negative reaction.

Please refer disclaimer Overleaf.

Identification Index

Tests	Group I Strains	Methyl Red	Voges Proskauer's	Urease	H_2S production	Citrate utilization	Lysine	ONPG	Lactose	Arabinose	Maltose	Sorbitol	Dulcitol
Most serotypes		+	-	-	+	+	+	-	-	+	+	+	+
Serotype Typhi		+	-	-	+	-	+	-	-	-	+	+	-
Serotype Choleraesuis subsp. choleraesuis		+	-	-	+	+	+	-	-	+	+	+	+
Serotype Paratyphi A		+	-	-	-	-	-	-	-	+	+	+	+
Serotype Gallinarum		+	-	-	+	-	+	-	-	V	+	-	+
Serotype Pullorum		+	-	-	+	-	+	-	-	+	-	-	-
S. serotype Typhimurium		+	-	-	+	+	+	-	-	+	+	+	V
S. choleraesuis subsp. arizonae		+	-	-	+	+	+	+	V	+	+	+	-
S. choleraesuis subsp. diarizonae		+	-	-	+	+	+	+	V	+	+	+	-
S. choleraesuis subsp. houtenae		+	-	-	+	+	+	-	-	+	+	+	-
S. choleraesuis subsp. indica		+	-	-	+	V	+	V	V	+	+	-	V
S. choleraesuis subsp. salamae		+	-	-	+	+	+	V	-	+	+	+	+

Tests		Methyl Red	Voges Proskauer's	Urease	H_2S production	Citrate utilization	Lysine	ONPG	Lactose	Arabinose	Maltose	Sorbitol	Dulcitol
S. enterica subsp. salamae	**Group II**	+	-	-	+	+	+	V	-	+	+	+	+
S. enterica subsp. arizonae	**Group IIIa**	+	-	-	+	+	+	+	V	+	+	+	-
S. enterica subsp. diarizonae	**Group IIIb**	+	-	-	+	+	+	+	V	+	+	+	-
S. enterica subsp. houtenae	**Group IV**	+	-	-	+	+	+	-	-	+	+	+	-
S. bongori	**Group V Strains**	+	-	-	+	+	+	+	-	+	+	+	+
S. enterica subsp. indica	**VI Strains**	+	-	-	+	V	+	V	V	+	+	-	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	Urease	—	Detects Urease activity	Orangish yellow	Pink	Orangish yellow
4	H_2S production	—	Detects H_2S production	Orangish yellow	**Black**	Orangish yellow
5	Citrate utilization	—	Detects capability of organism to utilize citrate as a sole carbon source	Green	**Blue**	Green
6	Lysine utilization	—	Detects Lysine decarboxylation	**Olive green to Light purple**	**Purple / Dark purple**	Yellow
7	ONPG	—	Detects β – galactosidase activity	Colourless	Yellow	Colourless
8	Lactose	—	Lactose utilization	Pinkish Red /Red	Yellow	Red / Pink
9	Arabinose	—	Arabinose utilization	Pinkish Red /Red	Yellow	Red / Pink
10	Maltose	—	Maltose utilization	Pinkish Red /Red	Yellow	Red / Pink
11	Sorbitol	—	Sorbitol utilization	Pinkish Red /Red	Yellow	Red / Pink
12	Dulcitol	—	Dulcitol 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 may 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 decarboxylation reaction, incubation up to 48 hours may be required.
4. At times organisms give contradictory 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 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.

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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