Technical Data

HiSalmonella™ Identification Kit KB011

Intended use

KB011 is a combination of 12 tests for identification of *Salmonella* species from clinical specimen and non clinical samples. It can also be used for validating known laboratory strains.

Kit Contains

1.Each kit contains 5/10/20 kits of KB011, sufficient material to perform 5/10/20 tests. Kit contains sterile media for MR test, Voges Proskauer's test, Urease production, H_2S production, Citrate utilization, Lysine utilization, ONPG tests and 5 different carbohydrate utilization tests - Lactose, Arabinose, Maltose,Sorbitol, Dulcitol

2. Methyl red reagent for MR Test (I007)
3. Barritt reagent A (R029) for VP test
4. Barritt reagent B (R030) for VP test
5. Technical product insert.
6. Result Interpretation Chart and Result Entry Datasheet.
7.Identification Index.

Material Required but not supplied

1. McFarland standard
2. Inoculation loops, pipettes
3. Enrichment medium / Isolation media

Direction

Preparation of inoculum :

1. Isolate the organism to be identified on a common medium like Nutrient Agar (M001) or Tryptone Soya Agar (M290).
2. Pick up a single isolated colony and inoculate in 5 ml BHI Broth (M210) and incubate at 35- 37°C for 4-6 hours until the inoculum turbidity is 0.1 OD at 620nm or 0.5 McFarland standard. Some organisms may require more than 6 hours of incubation. In this case incubate till the inoculum turbidity reaches 0.1 OD at 620nm.
3. 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 0.1 OD at 620nm.

Inoculation of the kit :

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

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 wherever required should be done at the end of incubation period that is after 18 - 24 hours.

Principle

Each KB011 Kit is a standardized calorimetric 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 colour change in the media that can be either interpreted visually or after addition of the reagents.

Type of specimen

Pure isolate from clinical specimen and non clinical sample

Specimen collection and handling

Refer direction

Warning and Precautions

In Vitro diagnostic Use. For professional use only. Read the label before opening the pack. Wear protective gloves/ protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Aseptic conditions should be maintained during inoculation and handling of the kits. Reagents should not come in contact with skin, eyes or clothing. Safety guidelines may be referred in individual safety data sheets.

Limitations

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 ± 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.
5.Erroneous false negative results may be obtained if the inoculum turbidity is less than 0.1 OD.
6.Results are more prominent if an enriched culture is used instead of a suspension.
7.It cannot be used directly for clinical specimens. The microorganisms to be identified have to be first isolated on appropriate isolation media. Only pure cultures should be used.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Sterile white opaque strip with 12 wells containing media for MR test, Voges Proskauer's test, Urease production, H_2S production, Citrate utilization, Lysine utilization, ONPG tests and 5 different carbohydrate utilization tests - Lactose, Arabinose, Maltose, Sorbitol, Dulcitol

Quantity of medium

0.8 ml of medium in each well.

Sterility Check

Passes release criteria

Interpretation of results :

Interpret results as per the standards given in the identification index. Addition of reagents in well no 1 and 2 should be done at the end of incubation period that is after 18 - 24 hours.

1.Methyl red Test : Well No. 1

- Add 2-3 drops of Methyl red indicator (I007).
- Positive test is indicated by a development of red colour.
- Yellowish orange colour indicates a negative reaction.

2.Voges-Proskaeur's Test: Well No.2

- Add 2-3 drops of Baritt reagent A (R029) and 1 drop of Baritt reagent B (R030).
- Positive test is indicated by a development of pinkish red colour in 5 - 10 minutes.
- No colour change or a copper colour (due to reaction of Reagent A and Reagent B) indicates a negative reaction.

3.Urease Test : Well No. 3

- Positive test is indicated by a colour change to pink colour.
- No colour change indicates a negative reaction.

4.H_2S production: Well No. 4

- Orangish yellow colour to black indicates a positive reaction
- No color change or slight yellowish brown indicates a negative reaction.

5. Citrate utilization: Well No. 5

- Positive test is indicated by a colour change to blue colour.
- Green or no colour change indicates a negative reaction.

6. Lysine utilization: Well No. 6

- Colour change to Purple / Dark Purple indicates positive reaction
- No colour change or yellow colour indicates negative reaction.

7. ONPG Test:Well No. 7

- Medium changes from colourless to yellow if the test is positive.
- Medium remains colourless if the test is negative.

8. Carbohydrate fermentation Test : Well No. 8-12

- Positive test is indicated by a colour change to yellow colour.
- Red or no colour change indicates a negative reaction.

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

KB011: HiSalmonella™ Identification Kit

Identification Index of various *Salmonella* species

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%)

Storage and Shelf Life

On receipt store between 2-8°C. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

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. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1.Bergey's Manual of Systematics of Archaea and Bacteria (BMSAB), 2015.
2.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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IVD ***In vitro* diagnostic medical device**

2°C 8°C **Storage temperature**

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CE Marking

Do not use if package is damaged

Do not re-use