Technical Data

HiStaph™ Identification Kit

KB004

Intended use

KB004 is a standardized, colorimetric identification system utilizing twelve conventional biochemical tests for identification and differentiation of genus *Staphylococcus* from clinical specimen and non clinical samples using pure isolate.

Kit contents

1.Each kit contains 5/10/20 kits of KB004, sufficient material to perform 5/10/20 tests. Kit sterile media for contains Voges Proskauer's, Phosphatase, ONPG, Urease production, Arginine utilization tests and 7 different carbohydrates utilization tests - Mannitol, Sucrose, Lactose, Arabinose, Raffinose, Trehalose, Maltose.

2.Technical product insert
5. Identification Index.
3. Baritt reagent A (R029) for VP test
4.Baritt reagent B (R030) for VP test
6.Result Interpretation Chart and Result Entry Datasheet.

Material Required but not supplied :

1.40% Sodium hydroxide solution for Phosphatase test.
2. McFarland standard
3. Inoculation loops, pipettes
4. 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 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.

Principle

KB004 is a standardized, colorimetric identification system utilizing twelve conventional biochemical tests. The tests are based on the principle of pH change and substrate utilization. On incubation, organisms undergo metabolic changes which are indicated as a colour change in the media that is either visible spontaneously or after addition of a reagent(1).

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 1.Voges Proskauer's, 2. Phosphatase, 3.ONPG, 4.Urease production, 5.Arginine utilization tests, and 7 different carbohydrates utilization tests -6.Mannitol, 7.Sucrose, 8.Lactose, 9.Arabinose, 10. Raffinose, 11. Trehalose and 12. Maltose

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.

Voges-Proskaeur's Test : Well No. 1

- 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.

Phosphatase Test : Well No. 2

- Add 1-2 drop of 40% Sodium hydroxide
- Positive test is indicated by development of bright pink colour within few seconds.
- Reagent remains colourless if the test is negative.

ONPG Test : Well No. 3

- Positive reaction will show yellow colour and negative reaction will show colourless.

Urease Test : Well No. 4

- Positive reaction will show pink colour and negative reaction will show orangish yellow colour.

Arginine utilization : Well no: 5

- Positive reaction will show purple / dark purple colour and negative reaction will show yellow colour.

Carbohydrate utilization: Well no: 6 to 12

- Positive reaction will show yellow colour and negative reaction will show red / pink colour.

Result Interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of medium	Positive reaction	Negative reaction
1	Voges Proskauer's	1-2 drops of Baritt reagent A and 1-2 drops of Baritt reagent 2	Detects acetoin production	Colourless/light yellow	Pinkish red	Colourless/ slight copper
2.	Alkaline phosphatase	1-2 drops of 40% NaOH	Detects ability of organism to produce sufficient phosphatase enzyme	Cream	Pink	Cream
3	ONPG	—	Detects galactosidase activity	Colourless	Yellow	Colourles
4	Urease	—	Detects Urease activity	Orangish-yellow	Pink	Orangish-yellow
5.	Arginine utilization	—	Detects Arginine decarboxylation	Olive green to Light	Purple / Dark purple	Yellow
6	Mannitol	—	Mannitol utilization	Pinkish Red / Red	Yellow	Red / Pink
7	Sucrose	—	Sucrose utilization	Pinkish Red / Red	Yellow	Red / Pink
8	Lactose	—	Lactose utilization	Pinkish Red / Red	Yellow	Red / Pink
9	Arabinose	—	Arabinose utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Raffinose	—	Raffinose utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Trehalose	—	Trehalose utilization	Pinkish Red / Red	Yellow	Red / Pink
12	Maltose	—	Maltose utilization	Pinkish Red / Red	Yellow	Red / Pink

Result Entry Datasheet

No.	Test	1	2	3	4	5	6	7	8	9	10
1	Voges-Proskaeur's										
2	Alkaline phosphatase										
3	ONPG										
4	Urease										
5	Arginine utilization										
6	Mannitol										
7	Sucrose										
8	Lactose										
9	Arabinose										
10	Raffinose										
11	Trehalose										
12	Maltose										

Identification Index of various *Staphylococcus* species

Tests	Voges Proskaeur's	Alkaline phosphatase	ONPG	Urease	Arginine utilization	Mannitol	Sucrose	Lactose	Arabinose	Raffinose	Trehalose	Maltose
S. aureus subsp. *aureus*	+	+	-	+w	+w	+	+	+	-	-	+	+
S. epidermidis	+	+	-	+	+w	-	+	v	-	-	-	+
S. haemolyticus	v	-	-	-	+	v	+	v	-	-	+	+
S. lugdunensis	+	-	-	v	-	-	+	+	-	-	+	+
S. saprophyticus	+	-	v	+	-w	v	+	v	-	-	+	+
S.schleiferi subsp. *coagulans*	+	+	nd	+	+	v	v	v	-	-	-	-
S.schleiferi subsp. *schleiferi*	+	+	v	-	+	-	-	-	-	-	v	-
S. arlettae	-	+	v	-	-	+	+	+	+	+	+	+
S. auricularis	v	-	v	-	v	-	v	-	-	-	+	+
S. capitis subsp. capitis	v	-	-	-	v	+	+	-	-	-	-	-
S. capitis subsp. ureolyticus	v	-	-	+	+	+	+	v	-	-	-	+
S. caprae	+	+	-	+	+	v	-	+	-	-	+	v
S. cohnii subsp. *cohnii*	v	-	-	-	-	v	-	-	-	-	+	v
S. cohnii subsp. *urealyticum*	v	+w	+	+	-w	v	-	+	-	-	+	v
S. hominis	v	-	-	+	v	-	+	v	-	-	v	+
S. pasteuri	v	-	-	+	v	v	+	v	-	-	+	v
S. simulans	-	+	+	+	+	+	+	-	-	-	v	-w
S. warneri	w	-	-	+	v	v	+	+	-	-	+	+
S. xylosus	+	v	+	+	-	v	+	v	-	-	+	+
S. caseolyticus	v	-	-	-	v	-	v	+	-	nd	v	+
S. carnosus	-	-	+	-	+	+	-	v	-	-	v	-
S. chromogens	+	+	-	v	+	+	+	+	-	-	+	v
S. delphini	–	+	nd	+	+	+	+	+	-	nd	-	+
S. equorum	-	+	v	+	-	+	+	v	+	-	+	v

S. felis	-	+	+	+	+	v	v	+	-	-	+	-
S. gallinarum	-	+	-w	+	-	+	+	v	+	+	+	+
S. hyicus	-	+	-	v	+	-	+	+	-	-	+	-
S. intermedius	-	+	v	+	v	v	+	v	-	-	+	+
S. kloosii	v	+	v	v	-	+	-	v	v	-w	+	v
S. lentus	-	+w	-	-	-	+	+	v	v	+	+	v
S. muscae	-	+	-	-	-	-	+	-	-	-	+	-
S. piscifermentans	-	+	v	+	+	v	v	v	-	-	+	v
S. sciuri	-	+w	-	-	-	+	+	-w	v	-	+	v
S. vitulus	-	-	-	-	-	+	+	-	-	-	v	-

S. hominis subsp. *novobiosepticus*	v	-	-	+	-	-	+	v	-	-	-	+
S. saprophyticus subsp. *bovis*	v	-	v	+	-	+	+	-	-	-	+	+
S. succinus	-	+	nd	+	-	nd	nd	+	nd	v	+	nd
S. carnosus subsp. utilis	nd	-	-	-	+	-	-	-	-	-	V	-
S. condimenti	nd	+	+	+	+	+	+w	+	-	-	+	-
S. lutrae	-	+	+	+	-	v	nd	+	nd	nd	+	+
S. sciuri subsp. *carnaticus*	-	V	-	-	-	+	+	v	v	-	+	V
S. sciuri subsp. *rodenti*	-	V	-	-	-	+	+	-	v	-	+	V
S. fleurettii	v	V	-	-	-	nd	+	-	v	-	+	+

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

+w = positive to weak
-w = reaction negative to weak reaction
Nd = not detected
V = variable, usually positive 11-89%
\+ = Positive (more than 90%)
\- = Negative (more than 90%)

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 clinical 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)
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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www.cepartner4u.eu

CE Marking

Do not use if package is damaged

Do not re-use

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