Technical Data

KB004 HiStaph™ Identification Kit

Introduction

KB004 is a biochemical test kit for identification and differentiation of genus *Staphylococcus*. The complete list of organisms that can be identified with this system is given in the identification index provided with the kit.

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.

Kit contents

1. Each kit contains sufficient material to perform 10 tests.
2. 10 kits of KB004.
3. Technical product insert.
4. Result Interpretation Chart and Result Entry Datasheet.
5. Identification Index.
6. Baritt reagent A (R029).
7. Baritt reagent B (R030).

Reagent required but not provided with the kit : 40% Sodium hydroxide solution for Phosphatase test.

Instructions for use

Note : KB004 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.

1. Preparation of inoculum :

- Isolate the organism to be identified on a common medium like Nutrient Agar (M001) or Soyabean Casein Digest Agar (M290). Pick up a single isolated colony and inoculate in 5 ml 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. Some organisms may require more than 6 hours of incubation. In this case incubate till the inoculum turbidity reaches 0.1OD at 620nm.
- 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 0.1OD at 620nm.

Note :
- Erroneous false negative results may be obtained if the inoculum turbidity is less than 0.1 OD.
- Results are more prominent if an enriched culture is used instead of a suspension.

2. Inoculation of the kit :

- Open the kit aseptically. Peel off the sealing foil.
- 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 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-Prokaeur's Test : Well No. 1

- Add 2-3 drops of Baritt reagent A and 1 drop of Baritt reagent B.
- 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.

Alkaline 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.

Result interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	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
2.	Alkaline phosphatase	1-2 drops of 40% NaOH	Detects ability of organism to produce sufficient phosphatase enzyme	Cream	Pink	Cream
3	ONPG	—	Detects β – galactosidase activity	Colourless	Yellow	Colourless
4	Urease	—	Detects Urease activity	Orangish-yellow	Pink	Orangish-yellow
5.	Arginine utilization	—	Detects Arginine decarboxylation	Olive green to Light purple	**Purple / Dark purple**	Yellow
6	Mannitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
7	Sucrose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
8	Lactose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
9	Arabinose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Raffinose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Trehalose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
12	Maltose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink

Please refer disclaimer Overleaf.

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	-w	+	+	+	+	+	+	-	-	-	v	-w
S. warneri	+	-	-	+	v	v	+	+	-	-	+	+
S. xylosus	v	v	+	+	-	v	+	v	-	-	+	+
S. caseolyticus	-	-	-	-	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. rodentium	-	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 reaction
-w = negative to weak reaction
Nd = not detected
V = variable, usually positive 11-89%

\+ = Positive (more than 90%)
\- = Negative (more than 90%)

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 ± 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.

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 disposal bag.

Storage and Shelf-life

Store at 2-8^{0}C. Shelf-life is 12 months.

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