Technical Data

KB008 HiNeisseria™ Identification Kit

Introduction

KB008 is a standardized test system for identification and differentiation of *Neisseria* species. *Neisseria* are gram negative cocci that inhabit the mucous membranes of humans and other animals. KB008 can be used for screening pathogenic *Neisseria* from nasopharyngeal, urethral, cervical exudates, rectal and pharyngeal specimens, blood and cerebrospinal fluid. 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 KB008 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, organisms undergo metabolic changes which are indicated by 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 KB008.
3. Technical product insert.
4. Result Interpretation Chart and Result Entry Datasheet.
5. Identification Index.
6. Sulphanilic acid (R015)
7. N,N-Dimethyl-1-Napthylamine Reagent (R009)
8. Barritt reagent A (R029) and Barritt reagent B (R030)
9. Gordon McCleod Reagent (R026)

Instructions for use

1. **Preparation of inoculum**
   - KB008 cannot be used directly on clinical specimens. The organism to be identified has to be first isolated on appropriate isolation medium and purified. Only pure cultures should be used.
   - Isolate the organism to be identified on a selective medium like Thayer Martin Medium Base (M413) or Chocolate Agar (M103).
   - Pick up a single isolated colony and inoculate in 5 ml Brain Heart Infusion Broth. Incubate at 35-37°C for 4-6 hours in 5 -10 % CO_2 and 70% humidity till the inoculum density is greater than or equal to 0.1 OD 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.
2. **Inoculation of the kit**
   - Open the kit aseptically.
   - 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 in 5 -10 % CO_2 and 70% humidity. 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. Following reagents are to be added to the respective wells.

Voges Proskaeur's Test : Well No. 3

- 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 change in colour (due to reaction of Baritt reagent A with Baritt reagent B) denotes a negative reaction.

Oxidase Test : Well No. 4

- Add 1-2 drops of Gordon -McCleod Reagent (R026).
- Positive test is indicated by development of purplish blue colour within 5-10 seconds, delayed slow reaction can be noted upto 60 seconds.
- No change in colour or delayed colour development after 60 seconds indicates a negative reaction.

Catalase Test : Well No. 5

- Scrape a loopful of growth from the surface of the well. Dip the loop in a small, clean test tube containing 3% Hydrogen Peroxide.
- 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% H_2O_2 has to be freshly prepared.

Nitrate Reduction Test : Well No. 6

- Add 1-2 drops of Sulphanilic acid (R015) and 1-2 drops of N,N-Dimethyl-1-Napthylamine Reagent (R009).
- Immediate development of pinkish red colour on addition of reagent indicates positive reaction.
- No change in colour 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	Urease	—	Detects Urease activity	Orangish yellow	Pink	Orangish yellow
2	ONPG	—	Detects β–galactosidase activity	Colourless	Yellow	Colourless
3	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
4.	Oxidase	1-2 drops of Gordon McCleod Reagent (R026)	Detects Cytochrome oxidase production	Colourless	Deep purple	No change in colour or purplish blue colour after 60 seconds
5.	Catalase	3% H_2O_2	Detects Catalase activity	Colourless	Effervescenence coming out from the loop	No effervescence seen
6	Nitrate reduction	1-2 drops of sulphanilic acid and 1-2 drops of N, N-Dimethyl-1-Napthylamine	Detects Nitrate reduction	Colourless	Pinkish red	Colourless
7	Glucose	—	Glucose utilization	**Pinkish Red /Red**	Yellow	**Red / Pink**
8.	Maltose	—	Maltose utilization	**Pinkish Red /Red**	Yellow	**Red / Pink**
9	Lactose	—	Lactose utilization	**Pinkish Red /Red**	Yellow	**Red / Pink**
10.	Sucrose	—	Sucrose utilization	**Pinkish Red /Red**	Yellow	**Red / Pink**
11.	Fructose	—	Fructose utilization	**Pinkish Red /Red**	Yellow	**Red / Pink**
12.	Mannose	—	Mannose utilization	**Pinkish Red /Red**	Yellow	**Red / Pink**

Identification Index of various *Neisseria species*

Tests	Urease	ONPG	Voges Proskauer's	Oxidase	Catalase	Nitrate reduction	Glucose	Maltose	Lactose	Sucrose	Fructose	Mannose
N. animalis	–	–	–	+	+	–	+	–	–	+	+weak	Nd
N. canis	–	–	–	+	+	+	–	–	–	–	–	–
N. cinerea	–	–	–	+	+	–	–	–	–	–	–	–
N. dinetrificans	–	–	–	+	+	–	+	–	–	+	+	+
N. elongata subsp. elongata	–	–	–	+	–	–	V	–	–	–	–	–
N. flavescens	–	–	–	+	+	–	–	–	–	–	–	–
N. gonorrhoeae	–	–	–	+	+	–	+	–	–	–	–	–
N. iguanae	–	–	–	+	+	–	V	–	–	–	Nd	Nd
N. lactamica	–	+	–	+	+	–	+	+	+	–	–	–
N. macacae	–	–	–	+	+	–	+	+	–	+	+	Nd
N. meningitidis	–	–	–	+	+	–	+	+	–	–	–	–
N. mucosa	–	–	–	+	–	+	+	+	–	+	+	–
N. perflava	–	–	–	+	+	–	+	+	–	+	+	Nd
N. polysaccharea	–	–	–	+	+	+	+	+	–	V	–	Nd
N. sicca	–	–	–	+	+	–	+	+	–	+	+	–
N. subflava	–	–	–	+	+	–	+	+	–	V	V	–
N. weaveri	–	–	–	+	+	–	–	–	–	–	Nd	Nd

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% positive)
Nd = Not detected.

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 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.
- Hydrogen peroxide is an extremely caustic solution so avoid exposure to skin. If H_2O_2 does get on the skin, immediately flood the area with 70% ethanol, 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 at 2-8°C. Shelf-life is 12 months.

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