KB014 HiAcinetobacter™ Identification Kit

Introduction

KB014 is identification system for Acinetobacter comprising of twenty four biochemical tests (Twelve tests in Part A and Twelve tests in part B). The tests include the IMViC group of tests, carbohydrate fermentation tests, amino acid hydrolysis tests and other tests. The complete list of organisms that can be identified with this system is given in the identification index provided with the kit.

Principle

KB014 is a standardized, colorimetric identification system utilizing twenty four 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 strips of KB014
3. Technical product insert
4. Result Interpretation Chart and Result Entry Datasheet
5. Identification Index
6. Kovac's reagent (R008)
7. Baritt reagent A (R029)
8. Baritt reagent B (R030)
9. TDA reagent (R036)
10. Alpha-Naphthylamine Solution (R009)
11. Sulphanilic Acid, 0.8% (R015)

Instructions for use

Note : KB014 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.

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 37°C for 4-6 hours until the inoculum turbidity is 0.1OD at 620nm or 0.5 McFarland standards. Some fastidious 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 2-3 ml 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 suspension.

Inoculation of the strip :

- Open the kit aseptically.
  Inoculate each well with 50 µl of the above inoculum by surface inoculation method.
- Alternatively, the strip can 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 in well no 1, 2, 7 & 10 of part A should be done at the end of incubation period that is after 18 – 24 hours.

PART A :

Well No. 1: Indole test :

- Add 1-2 drops of Kovac's reagent to well no 1.
- Positive test is indicated by development of reddish pink colour within 10 seconds.
- Absence of reddish pink colour development denotes a negative reaction.

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

- Add 2-3 drops of Baritt reagent A and 1-2 drops of Baritt reagent B to well no 2.
  Reddish pink 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.

Well No. 3: Citrate Test :

- No reagent to be added.
- Colour of the medium changes from its original green to blue colour if the test is positive.
- Medium remains green if the test is negative.

Well No. 4: Lysine Test :

- No reagent to be added.
- Colour of the medium changes from its original olive green to purple colour if the test is positive.
- Medium turns yellow if the test is negative.

Well No. 5: Ornithine Test :

- No reagent to be added.
- Colour of the medium changes from its original olive green to purple colour if the test is positive.
- Medium turns yellow if the test is negative.

Well No. 6: Arginine Test :

- No reagent to be added.
- Colour of the medium changes from its original olive green to purple colour if the test is positive.
- Medium turns yellow if the test is negative.

Well No. 7: Nitrate Reduction Test :

- Add 1-2 drops of sulphanilic acid and 1-2 drps of Alpha-Naphthylamine Solution to well no 7.
- Positive test is indicated by development of pinkish red colour.
- Absence of pinkish red colour development denotes a negative reaction.

Well No. 8: Malonate Test :

- No reagent to be added.
- Colour of the medium changes from its original light green to blue colour if the test is positive.
- Medium remains light green if the test is negative.

Well No. 9: Urease Test :

- No reagent to be added.
- Colour of the medium changes from its original orangish yellow to pink colour if the test is positive.
  Medium remains orangish yellow if the test is negative

Well No. 10: Phenylalanine Deamination Test :

- Add 2-3 drops of TDA reagent to well no 10.
- Positive test is indicated by development of green colour.
  Absence of green colour development denotes a negative reaction.

Well No. 11: H2S Production Test :

- No reagent to be added.
- Colour of the medium changes from its original orangish yellow to black colour if the test is positive.
- Medium remains orangish yellow if the test is negative.

Well No. 12: ONPG Test :

- No reagent to be added.
- Colour of the medium changes from its original colourless to yellow colour if the test is positive.
- Medium remains colourless if the test is negative.

Part B :

Well No 1 to12: Carbohydrate fermentation test :

- No reagent to be added.
- Colour of the medium changes from its original red to yellow colour due to acid production indicating a positive reaction.
- Medium remains red in colour if the test is negative.

Biochemical reactions of KB014 PART A

Well No	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Indole	1-2 drops of Kovac's reagent	Detects deamination of tryptophan	Colourless	Reddish pink	Colourless
2	Voges-Proskauer	1-2 drops of Baritt reagent A and 1-2 drops of Barrit reagent B	Detects acetoin production	Colourless	Pinkish red	Colourless/Slight copper
3	Citrate utilization	-	Detects capability of organism to utilize citrate as a sole carbon source.	Yellowish green	Blue	Yellowish green
4	Lysine utilization	-	Detects Lysine decarboxylation	Olive green	Purple	yellow
5	Ornithine utilization	-	Detects Lysine decarboxylation	Olive green	Purple	yellow
6	Arginine utilization	-	Detects Arginine decarboxylation	Olive green	Purple	yellow
7	Nitrate reduction	1-2 drops of sulphanilic acid and 1-2 drops of Alpha-Naphthylamine Solution	Detects nitrate reduction	Colourless	Pinkish red	Colourless
8	Malonate	-	Detects capability of organism to utilize sodium malonate as a sole carbon source	Light green	Blue	Light green
9	Urease	-	Detects urease activity	Orangish yellow	Pink	Orangish yellow
10	Phenylalanine deamination	2-3 drops of TDA reagent	Detects phenylalanine deamination activity	Colourless	Green	Colourless
11	H_2S Production	-	Detects H_2S Production	Orangish yellow	Black	Orangish yellow
12	ONPG	-	Detects Beta-galactosidase activity	Colourless	yellow	Colourless

Result interpretation chart

Well No	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Glucose	-	Glucose utilization	Red	Yellow	Red/Pink
2	Mannitol	-	Mannitol utilization	Red	Yellow	Red/Pink
3	Xylose	-	Xylose utilization	Red	Yellow	Red/Pink
4	Inositol	-	Inositol utilization	Red	Yellow	Red/Pink
5	Sorbitol	-	Sorbitol utilization	Red	Yellow	Red/Pink
6	Rhamnose	-	Rhamnose utilization	Red	Yellow	Red/Pink
7	Sucrose	-	Sucrose utilization	Red	Yellow	Red/Pink
8	Lactose	-	Lactose utilization	Red	Yellow	Red/Pink
9	Arabinose	-	Arabinose utilization	Red	Yellow	Red/Pink
10	Adonitol	-	Adonitol utilization	Red	Yellow	Red/Pink
11	Raffinose	-	Raffinose utilization	Red	Yellow	Red/Pink
12	Salicin	-	Salicin utilization	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 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. At times organisms give conflicting result because of mutation or the media used for isolation, cultivation & maintenance.
4. The identification index has been compiled from standard references & 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 & handling of the strips.
- Reagents should not come in contact with skin, eyes or clothing.

Disposal of used material :

After use, strips and the instruments used for isolation & 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 between 2-8^{0}C. Shelf-life is 12 months.

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