

KB019 Hi24™ Nonfermenters Identification

Intended Use :

KB019 is a comprehensive test system that can be used for the identification of non fermenters. Organisms belonging to non fermenters are gram negative and non sporulating and are isolated from clinical samples.

Hi24™ Nonfermenters Identification kit can be used for screening pathogenic organisms from urine, enteric specimens and other relevant clinical samples. Organism isolated includes the genus *Acinetobacter*, *Bordetella*, *Burkholderia*, *Legionella*, *Moraxella*, *Pseudomonas* and *Stenotrophomonas*. 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 Hi24™ Nonfermenters Identification kit is a standardized colorimetric identification system utilizing twelve conventional biochemical tests and twelve 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 as a colour change in the media that can be either interpreted visually or after addition of the reagent.

Kit contents :

1. Each kit contains sufficient material to perform 10 tests
2. 10 strips of KB019
3. Technical product insert
4. Result Interpretation Chart and Result Entry Datasheet
5. Identification Index

Type of specimen

Clinical samples : Blood, Urine, Faeces, Enteric, Nosocomial and other relevant clinical samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines.

Warning and Precautions :

In Vitro diagnostic Use only. Read the label before opening the kit. 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. 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. Safety guidelines may be referred in safety data sheets.

Limitations :

1. KB019 cannot be used directly on clinical specimens. The organisms to be identified have to be first isolated and purified. Only pure cultures should be used.
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.
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.

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.

Instructions for use Interpretation of Results :

Interpret results as per the standards given in the Result Interpretation Chart. Addition of reagents in well nos 1, 5, 8 and 12 should be done at the end of incubation period, that is after 18 - 24 hours. Following reagents to be added to the respective wells.

Indole Test : Well No.1

Add 1-2 drops of Kovac's reagent (R008). Development of reddish pink colour within 10 seconds indicates positive reaction. Reagent remains pale coloured if the test is negative.

Nitrate Reduction Test : Well No.5

Add 1-2 drops of Sulphanilic acid (R015) and 1-2 drops of α-Naphthylamine Solution (R009) Immediate development of pinkish red colour on addition of reagent indicates positive reaction No change in colour indicates a negative treatment.

Phenylalanine Deamination Test – Well No.8

Add 2-3 drops of TDA Reagent. Development of dark green colour within one minute indicates positive reaction. No change in colour denotes a negative reaction.

Oxidase Test – Well No.12

Add 1-2 drops of Gordon McLeod Reagent.

Development of purplish blue colour within 5-10 seconds indicates positive reaction. Delayed positive reaction upto 60 seconds may be noted.

No change in colour or delayed positive reaction upto 60 seconds denotes a negative reaction.

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 .

Disposal of used material :

After use, kits and the materials 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.

Instructions for use

Preparation of Inoculum

- Isolate the organism to be identified on a common medium like Nutrient Agar (M001/ M1274) or Brain Heart Infusion Agar (M211).
- Pick up a single well isolated colony and inoculate in 5ml Brain Heart Infusion broth 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.
- Alternatively, a homogeneous suspension made in 2-3 ml sterile saline can be used for inoculation.
- The density of the suspension should be adjusted 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.
- Results are more prominent when enriched culture instead of suspension.

Inoculation of the kit

- Open the kit aseptically. Peel off the sealing tape.
- Inoculate each well with 50 µl of the above inoculum by surface inoculation method.
- Alternatively the kit can be inoculated by stabbing each individual well with a loopful of inoculum.

Incubation :

- Temperature of incubation : 35 - 37°C. Duration of incubation : 24-48 hours.

Storage and Shelf life

On receipt, store at 2-8°C. Shelf life is 12 months. Product performance is best if used within stated expiry period.

Sr. No.	Organisms	Indole	Esculin	Citrate	Arginine	Nitrate	ONPG	Alkaline phosphatase	Phenyl alanine	Urease	Cetrimide	Lysine	Oxidase
1.	Achromobacter Group A	-	-	-	-	+	-	-	-	+	-	-	+
2.	Achromobacter Group B	-	+	-	-	+	+	+	-	+	-	-	+
3.	Achromobacter Group C	-	-	-	-	+	+	-	-	+	-	-	+
4.	Achromobacter Group D	-	-	-	-	+	-	-	-	+	-	-	+
5.	Achromobacter Group E	-	+	-	+	+	+	-	-	+	-	-	+
6.	Achromobacter Group F	-	+	-	-	+	-	-	-	+	-	-	+
7.	Achromobacter xylosoxidans	-	-	+	-	+	-	-	-	-	+	-	+
8.	Acinetobacter calcoaceticus	-	-	+	-	-	-	-	-	-	-	-	-
9.	Acinetobacter lwoffii	-	-	-	-	-	-	-	-	-	-	-	-
10.	Agrobacterium rhizogenes	-	+	-	-	-	+	-	-	-	-	-	+
11.	Agrobacterium rubi	-	+	-	-	-	+	-	-	+	-	-	+
12.	Agrobacterium tumefaciens	-	+	-	-	-	-	+	-	+	-	-	+
13.	Agrobacterium yellow group	-	+	-	-	-	-	+	-	-	-	-	+
14.	Alkaligenes denitrificans	-	-	+	-	+	-	+	-	-	+	-	+
15.	Alkaligenes faecalis	-	-	+	-	-	-	-	-	-	+	-	+
16.	Alteromonas putrefaciens	-	-	-	-	+	-	+	-	-	-	-	+
17.	Bordetella bronchiseptica	-	-	+	-	+	-	-	-	+	-	-	+
18.	Bordetella parapertussis	-	-	-	-	-	-	-	-	+	-	-	-
19.	Brahmella/ M.nontliquefaciens/ Neisseria	-	-	-	-	-	-	-	-	-	-	-	+
20.	Brucella spp.	-	-	-	-	+	-	-	-	+	-	-	+
21.	Burkholderia. cepacia	-	-	+	-	-	-	+	-	+	+	+	+
22.	CDC Group IIf	+	-	-	-	-	-	-	-	-	-	-	+
23.	CDC Group Iij	+	-	-	-	-	-	-	-	+	-	-	+
24.	CDC Group IVe	-	-	-	-	+	-	-	-	+	-	-	+
25.	CDC Group Ve type 1	-	+	+	+	+	+	-	-	+	-	-	-
26.	CDC Group Ve type 2	-	-	+	-	-	-	-	-	+	+	-	-
27.	Eikenella corrodens	-	-	-	-	+	-	+	-	-	-	+	+
28.	Flavobacterium breve	+	-	-	-	-	-	-	-	-	-	-	+
29.	Flavobacterium meningosepticum	-	-	-	-	-	+	-	-	-	-	-	+
30.	Flavobacterium multivorum	-	-	-	-	-	+	-	-	+	-	-	+
31.	Flavobacterium odoratum	-	-	-	-	-	-	-	-	+	-	-	+
32.	Flavobacterium species Group Iib	+	+	-	-	-	-	-	-	-	-	-	+
33.	Flavobacterium spiritivorum	-	+	-	-	-	+	-	-	+	-	-	+
34.	Flavobacterium thalophilum	-	+	-	-	+	+	+	-	+	-	-	+
35.	Janthinobacterium lividum	-	+	-	-	+	-	+	-	-	-	-	+
36.	Kingella denitrificans	-	-	+	-	+	-	+	-	-	-	-	+
37.	Kingella indologenes	-	-	-	-	-	-	+	-	-	-	-	+
38.	Kingella kingae	+	-	-	-	-	-	+	-	-	-	-	+
39.	Moraxella anatipestifer	-	-	-	-	-	-	-	-	+	-	-	+
40.	Moraxella osloensis	-	-	-	-	-	-	-	-	-	-	-	+
41.	Moraxella phenylpyruvica	-	-	-	-	+	-	-	-	+	-	-	+
42.	Moraxella proteolytic group	-	-	-	-	+	-	-	-	-	-	-	+
43.	Moraxella saccharolytica	-	+	-	-	+	-	+	-	-	-	-	+
44.	Moraxella urethralis	-	-	-	-	-	-	-	-	-	-	-	+
45.	Pseudomonas acidovorans	-	-	+	-	+	-	-	-	-	-	-	+
46.	P. aeruginosa	-	-	+	+	+	-	-	-	+	+	-	+
47.	P. alcaligenes	-	-	+	-	+	-	-	-	-	+	-	+
48.	P. diminuta	-	-	-	-	-	-	-	-	-	-	-	+
49.	P. fluorescens	-	-	+	+	-	-	-	-	-	+	-	+
50.	P. fragi	-	-	+	+	-	-	+	-	-	+	-	+
51.	P. lemoignei	-	-	-	-	-	-	-	-	-	-	-	+
52.	P.mallei	-	-	-	+	+	-	-	-	-	-	-	-
53.	P.maltophilia	-	+	-	-	+	-	+	-	-	-	+	-
54.	P. mendocina	-	-	+	+	+	-	+	-	+	+	-	+
55.	P. paucimobilitis	-	+	-	-	-	+	+	-	-	-	-	+
56.	P. pickettii	-	-	+	-	+	-	-	-	+	-	-	+
57.	P.pseudoalcaligenes	-	-	-	-	+	-	-	-	-	+	-	+
58.	P. pseudomallei	-	-	+	+	+	-	+	-	-	-	-	+
59.	P. putida	-	-	+	+	-	-	-	-	-	+	-	+
60.	P.stutzeri	-	-	+	-	+	-	-	-	-	-	-	+
61.	P.taetrolens	-	-	+	+	-	-	-	-	+	+	-	+
62.	P. testosteroni	-	-	+	-	-	-	+	-	-	-	-	+
63.	P.vesicularis	-	-	-	-	-	-	+	-	-	-	-	+
64.	Rhizobium meliloti	-	+	-	-	-	+	-	-	+	-	-	+
65.	Xanthomonas hyacinthi	-	+	-	-	-	-	-	-	-	-	-	+
66.	Xanthomonas spp. (not hyacinthi)	-	+	+	-	-	-	-	-	-	-	-	+

Sr. No.	Organisms	Lactose	Maltose	Mannitol	Trehalose	Sucrose	Arabinose	Cellobiose	Fructose	Galactose	Glucose	Inositol	Xylose
1.	Achromobacter Group A	-	+	+	+	+	+	+	+	+	+	+	+
2.	Achromobacter Group B	-	+	+	+	+	+	+	+	+	+	+	+
3.	Achromobacter Group C	+	+	-	+	+	+	+	+	+	+	+	+
4.	Achromobacter Group D	-	-	+	-	-	+	-	+	+	+	+	+
5.	Achromobacter Group E	-	+	-	+	+	+	+	+	-	+	+	+
6.	Achromobacter Group F	-	+	+	+	+	+	+	+	-	+	-	+
7.	Achromobacter xylooxidans	-	-	-	-	-	-	-	-	-	+	-	+
8.	Acinetobacter calcoaceticus	+	-	-	-	-	+	+	-	+	+	-	+
9.	Acinetobacter Iwoffii	-	-	-	-	-	-	-	-	-	-	-	wp
10.	Agrobacterium rhizogenes	+	+	+	+	+	+	+	+	+	+	-	+
11.	Agrobacterium rubi	+	+	+	+	+	+	+	+	-	+	+	+
12.	Agrobacterium tumefaciens	+	+	+	+	+	+	+	+	-	+	+	+
13.	Agrobacterium yellow group	+	+	-	+	+	+	+	+	+	+	+	+
14.	Alkalicoccus denitrificans	-	-	-	+	-	-	-	-	-	-	-	-
15.	Alkalicoccus faecalis	-	-	-	-	-	-	-	-	-	-	-	-
16.	Alteromonas putrefaciens	-	-	-	+	-	-	-	-	-	-	-	-
17.	Bordetella bronchiseptica	-	-	-	+	-	-	-	-	+	-	-	-
18.	Bordetella parapertussis	-	-	-	+	-	-	-	-	-	-	-	-
19.	Brahmella/ M.nonliquefaciens/ Neisseria	-	-	-	+	-	-	-	-	-	-	-	-
20.	Brucella spp.	-	-	-	+	-	-	-	-	+	-	-	-
21.	CDC Group III	-	-	-	+	-	-	-	-	+	-	-	-
22.	CDC Group Iij	-	-	-	+	-	-	-	-	-	-	-	-
23.	CDC Group IVe	-	-	-	-	-	-	-	-	-	-	-	-
24.	CDC Group Ve type 1	-	+	+	+	-	+	-	+	-	+	+	+
25.	CDC Group Ve type 2	-	+	+	+	-	+	-	+	-	+	+	+
26.	Eikenella corrodens	-	-	-	-	-	-	-	-	-	-	-	-
27.	Flavobacterium breve	-	+	-	-	-	-	-	-	+	+	-	-
28.	Flavobacterium meningosepticum	+	+	+	+	-	-	-	+	-	+	-	-
29.	Flavobacterium multivorum	+	+	-	+	+	+	+	+	-	+	-	+
30.	Flavobacterium odoratum	-	-	-	-	-	-	-	-	-	-	-	-
31.	Flavobacterium species Group lib	+	+	-	+	-	-	-	+	+	+	-	-
32.	Flavobacterium spiritivorum	-	+	+	+	+	+	+	+	-	+	-	+
33.	Flavobacterium thalophilum	-	+	-	+	+	+	+	+	-	+	-	+
34.	Janthinobacterium lividum	+	+	+	-	+	+	+	+	-	+	+	+
35.	Kingella denitrificans	-	-	-	-	-	-	-	-	+	-	-	-
36.	Kingella indologenes	-	-	-	-	-	-	-	-	-	-	-	-
37.	Kingella kingae	-	-	-	-	-	-	-	-	-	-	-	-
38.	Moraxella anatipestifer	-	-	-	-	-	-	-	-	+	-	-	-
39.	Moraxella osloensis	-	-	-	-	-	-	-	-	-	-	-	-
40.	Moraxella phenylpyruvica	-	-	-	-	-	-	-	-	-	-	-	-
41.	Moraxella proteolytic group	-	-	-	-	-	-	-	-	+	-	-	-
42.	Moraxella saccharolytica	-	-	-	-	+	-	+	+	-	+	-	-
43.	Moraxella urethralis	-	+	-	-	-	-	-	-	-	-	-	-
44.	Pseudomonas acidovorans	-	-	+	-	-	-	-	+	-	-	-	-
45.	P. aeruginosa	-	-	+	-	-	+	-	+	+	+	-	+
46.	P. alcaligenes	-	-	-	-	-	-	-	-	-	-	-	-
47.	P. cepacia	+	+	+	+	+	+	+	+	-	+	+	+
48.	P. diminuta	-	-	-	-	-	-	-	-	-	-	-	-
49.	P. fluorescens	-	-	+	+	-	+	-	+	-	+	+	+
50.	P. fragi	-	+	-	+	+	+	+	+	+	+	-	+
51.	P. lemoignei	-	-	-	-	-	-	-	-	-	-	-	-
52.	P. mallei	+	-	+	+	-	+	-	-	-	+	+	-
53.	P. maltophilia	-	+	-	-	-	-	-	-	-	+	-	-
54.	P. mendocina	-	-	-	-	-	-	-	+	-	+	-	+
55.	P. paucimobilis	-	+	-	+	+	+	+	+	-	+	-	+
56.	P. pickettii	-	+	-	-	-	+	+	+	+	+	-	+
57.	P. pseudoalcaligenes	-	-	-	-	-	-	-	+	-	-	-	-
58.	P. pseudomallei	+	+	+	+	-	+	+	+	-	+	+	-
59.	P. putida	-	-	-	-	-	+	-	+	+	+	-	+
60.	P. stutzeri	-	+	-	-	-	-	-	+	-	+	-	+
61.	P. taetrolens	+	+	+	-	-	+	+	+	-	+	+	+
62.	P. testosteroni	-	-	-	-	-	-	-	-	+	-	-	-
63.	P. vesicularis	-	+	-	-	-	-	+	-	-	+	-	-
64.	Rhizobium meliloti	+	+	+	+	+	+	+	+	-	+	+	+
65.	Xanthomonas hyacinthi	-	+	-	-	-	+	+	+	+	+	-	-
66.	Xanthomonas spp. (not hyacinthi)	-	+	-	+	+	+	+	+	-	+	-	+

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 = 11-89% Positive

Nd = No data available.

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 Indole Reagent	Detects deamination of Tryptophan	Colourless to pale yellow	Pinkish red	Colourless
2	Esculin hydrolysis		Detects esculin hydrolysis	Cream	Black	Cream
3	Citrate utilization		Detects capability of organism to use citrate as sole carbon source	Green	Blue	Green
4	Arginine		Detects Arginine decarboxylation	Olive green to light purple	Purple / Dark purple	Yellow
5	Nitrate reduction	1-2 drops of sulphanic acid and 1-2 drops of α -naphthylamine solution	Detects Nitrate reduction	Colourless	Pinkish red	Colourless
6	ONPG		Detects β -galactosidase activity	Colourless	Yellow	Colourless
7	Alkaline phosphatase	On addition of alkali	Detects presence of phosphatase	Colourless	Pink	Colourless
8	Phenylalanine deamination	2-3 drops of TDA Reagent	Detects phenylalanine deamination activity	Colourless	Green	Colourless
9	Urease		Detects Urease activity	Orangish yellow	Pink	Orangish yellow
10	Cetrimide tolerance		Detects tolerance of cetrimide	Colourless to yellow		
11	Lysine		Detects Lysine decarboxylation	Olive green to light purple	Purple / Dark purple	Yellow
12	Oxidase	2-3 drops of Gordan Mcheocl Reagent	Detects presence of Oxidase	Colourless		

Strip II

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative Reaction
1	Lactose		Lactose utilization	Green	Yellow	Green
2	Maltose		Maltose utilization	Green	Yellow	Green
3	Mannitol		Mannitol utilization	Green	Yellow	Green
4	Trehalose		Trehalose utilization	Green	Yellow	Green
5	Sucrose		Sucrose utilization	Green	Yellow	Green
6	Arabinose		Arabinose utilization	Green	Yellow	Green
7	Cellobiose		Cellobiose utilization	Green	Yellow	Green
8	Fructose		Fructose utilization	Green	Yellow	Green
9	Galactose		Galactose utilization	Green	Yellow	Green
10	Glucose		Glucose utilization	Green	Yellow	Green
11	Inositol		Inositol utilization	Green	Yellow	Green
12	Xylose		Xylose utilization	Green	Yellow	Green

Strip II

	1	2	3	4	5	6	7	8	9	10
1	Indole									
2	Esculin hydrolysis									
3	Citrate utilization									
4	Arginine									
5	Nitrate reduction									
6	ONPG									
7	Alkaline phosphatase									
8	Phenylalanine deamination									
9	Urease									
10	Cetrimide tolerance									
11	Lysine									
12	Oxidase									
13	Lactose									
14	Maltose									
15	Mannitol									
16	Trehalose									
17	Sucrose									
18	Arabinose									
19	Cellobiose									
20	Fructose									
21	Galactose									
22	Glucose									
23	Inositol									
24	Xylose									



Disclaimer :

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