HiBio-ID™

TEST KITS

Available
With
reagents required
for each kit

- Simple & accurate bacterial identification kit.
- A standardized, miniaturized version of Conventional Tube Biochemical methods.

A combination of tests

- IMViC
- Carbohydrate utilization
- Amino acid utilization
- Phenylalanine deamination
- Urea utilization
- Malonate utilization
- ONPG test
- Glucoronidase test
- Nitrate reduction
- Esculin hydrolysis
- H_2S production
- Motility test
- Catalase test
- Oxidase test
- Salt tolerance test
- Pyrrolidonyl-β-Naphthylamide hydrolysis (PYR)
- Alkaline phosphatase

ISO 9001-2008 CERTIFIED

HiMediaLaboratories™

HiMedia Laboratories Pvt. Limited

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For life is precious

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HiBio-ID™ TEST KITS

Introduction

HiMedia provides a range of Biochemical Identification test kit (KB001 to KB012) and Motility Test kit (KBM001 to KBM003) involving single step procedure of inoculation which leads to final identification of test organism being studied. Each Biochemical Identification test kit is a standardized colorimetric identification system utilizing conventional biochemical tests and 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 interpreted visually or after addition of a reagent.

Kit contents

1. Biochemical Identification test kit.
2. Technical product insert.
3. Result Interpretation Chart and Result Entry Datasheet.
4. Identification Index.

   Note : *The identification index has been compiled from standard references and results of tests carried out in the laboratory.*
5. Reagents for the kit, wherever it is required.

General Instructions

Preparation of inoculum

- The organisms to be identified have to be first isolated and purified. Only pure cultures should be used. Clinical specimens cannot be directly used for isolation.
- Isolate the organism to be identified on a common medium like Nutrient Agar (M001) or a suitable differential medium as per use.
- Pick up a single isolated colony and inoculate in 5 ml Brain Heart Infusion Broth (M210) and incubate at 35-37°C for 4-6 hours until the inoculum turbidity is as prescribed in the individual product insert. Some fastidious organisms may require more than 6 hours of incubation. In this case incubate till the inoculum turbidity reaches the prescribed OD.
- 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 adjusted as per the instructions in individual product insert.
- Follow the instructions as per individual product insert
- Note the result in the Result Entry Datasheet after comparing with the result interpretation chart.

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 kit

- Open the kit aseptically. Peel off the sealing foil. Inoculate each well of KB kit 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.

  In case of KBM, inoculate all the wells (except well number 2) by stab inoculation.

Incubation :

KBM001, KBM002 and KB001 to KB012 is incubated at 35 ± 2°C for 18-24 hrs except KB006 at room temperature (22-25°C) for 48-72 hrs and KBM003 at room temperature (22-25°C) for 24-48 hrs.

Reagent :

Wherever necessary, the reagents should be added. For more details refer Result Interpretation chart.

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.

Principle of Biochemical Tests involved

Alkaline Phosphatase Test

This test detects the ability of microorganism to produce sufficient phosphatase enzyme. Phosphatase production is determined by liberation of phenolphthalein. The liberated phenolphthalein reacts with alkali (40% NaOH) to give a bright pink colour.

Amino acid decarboxylation test

(Lysine, Ornithine, Arginine)

The medium for this test contains Bromocresol purple as pH indicator. When carbohydrate present in the medium is utilized, pH is lowered due to acid production changing the colour of medium to yellow. The acid produced stimulates decarboxylase enzyme. The formation of amine due to this reaction increases the pH of the medium, changing the colour of the indicator from olive green or light purple to purple or dark purple. Negative reaction is indicated by development of yellow colour.

In case of Lysine and Ornithine decarboxylation, incubation upto 48 hours may be required.

Carbohydrate utilization

Specific carbohydrates are added to basal media which contains phenol red as indicator. On fermentation of carbohydrate, acid is liberated which lowers down the pH of medium and this change of colour is indicated by pH indicator dye. Positive test is indicated by colour change to yellow due to acid reaction. No change in colour or red/pink colour indicates negative reaction.

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.

Catalase

Organisms producing catalase have the ability to breakdown hydrogen peroxide to nascent oxygen. This is noted as a quick effervescence or bubbles on the surface of the medium. This can be detected by addition of one drop of 3% hydrogen peroxide to the growth to be tested for catalase activity. One drop of H_2O_2 is added to the growth to be tested for catalase activity.

Citrate Test

The medium contains sodium citrate as sole source of carbon, bromothymol blue as indicator and inorganic ammonium salts. The organism that is capable of utilizing citrate as its sole source of carbon also utilizes the ammonium salts as its sole nitrogen source. Ammonium salts are broken down to ammonia (NH_3) with resulting alkalinity. The indicator therefore turns blue from green indicating alkaline condition.

Esculin Hydrolysis Test

Esculin is substituted glucoside that can be hydrolyzed by certain bacteria to yield glucose and esculetin. The latter combines with ferric ions in the medium to form a black coloured complex.

Glucuronidase

The enzyme glucuronidase cleaves the substrate x- glucoronide in the medium. The microorganism absorbs the substrate and the intracellular glucuronidase splits the bond between the chromophore and the glucuronide. The released chromophore gives bluish green colouration to the colonies or growth.

H_2S Production Test

All members of *Enterobacteriaceae* are capable of producing various amounts of H_2S. Microorganisms are capable of enzymatically liberating sulfur from sulfur-containing amino acids or inorganic sulfur compounds. The hydrogen sulfide released reacts with ferric ions or lead acetate to yield ferrous sulphide or lead sulphide, which are insoluble black precipitates. Blackening of medium indicates positive reaction.

Indole Test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophane to indole which accumulates in the medium. On addition of Kovac's Indole reagent (R008), development of reddish pink colour within 10 seconds indicates positive test.

Malonate Test

Malonate test medium contains Bromothymol blue as indicator. Sodium malonate is the carbon source and ammonium sulphate is the nitrogen source. Organisms, which are able to utilize malonate, release sodium hydroxide. The resulting alkaline conditions cause the indicator to change from light green to blue. Malonate-negative organisms do not cause any change in the colour of the medium.

Methyl Red Test

The methyl red test is based on the use of a pH indicator, methyl red to determine the hydrogen ion concentration (pH) when an organism utilizes glucose. On addition of one drop of methyl red indicator (I007) at the end of the period of incubation, if the colour changes to distinct red it indicates positive test. Whereas if the reagent turns yellow or yellowish orange, it indicates negative test.

Sterile KB002 before inoculation

Culture inoculation

Incubation

Adding reagent to read the cultural response

KB002 results

Motility Test

In case of motile organisms, after stab inoculation in first well and respective incubation time and temperature conditions, movement of growth from first to second well is observed along with/without biochemical reactions change, if any.

Nitrate reduction

Gram-negative bacilli vary in their ability to reduce nitrates. Members of *Enterobacteriaceae* characteristically reduce nitrate to nitrite which reacts with sulphanilic acid (R015) and N, N-dimethyl-1-napthylamine reagent (R009) to produce pinkish red colour. Nitrate Reagent Discs (DD042) can also be used for this test. Pinkish red to red colouration on discs indicates positive test.

ONPG Test

Two enzymes, Permease and β-galactosidase are required for Lactose fermentation. True non-lactose fermenters are devoid of both enzymes, however some organisms may lack permease but posses the enzyme β-galactosidase. ONPG (o-nitrophenyl-β-D-galactopyranoside) is structurally similar to lactose. In the presence of β-galactosidase, ONPG is cleaved into galactose and o-nitrophenol, a yellow compound. Since members of *Enterobacteriaceae* are routinely grouped according to their abilities to ferment lactose, the ONPG test is especially useful in rapidly identifying cryptic lactose fermentation. Development of a yellow colour when ONPG Discs (DD008) is placed on 8 - 24 hours growth and incubated further for a minimum of 1 hour at 35-37^{0}C, indicates positive reaction.

Oxidase

This test depends on the presence in bacteria of certain oxidases that will catalyse the transport of electrons between electron donors in the bacteria and a redox dye tetramethyl-p-phenylenediamine. The dye is reduced to a deep purple colour.

The test is carried out by touching oxidase discs (DD018)/ addition of Gordon-McLeod Reagent (R026) to the growth surface. Development of purple colour within 5-10 seconds indicates positive reaction, delayed positive reaction appears in 10-60 seconds whereas no change in colour is considered as negative reaction.

Phenylalanine Deamination

This test detects the ability of an organism to oxidatively deaminate phenylalanine with production of phenylpyruvic acid which reacts with ferric salts to give a green colour.

On addition of TDA reagent (R036) to the medium after incubation appearance of green colour indicates a positive reaction. No change in colour indicates negative reaction. Interpret the results within 5 minutes after addition of reagent as the green colour fades quickly.

PYR Test

This test detects the ability of Gram positive bacteria especially Group A, *Streptococci pyogenes* and most Group D *Enterococcus* species to produce pyrrolidonyl arylamidase enzyme. It hydrolyses the substrate, L-pyrrolindonyl β - naphthylamine to from free colourless L- Pyrrolidone and free β - naphthylamine.

PYR reagent (p-Dimethylaminocinnamaldehyde, DMACA) (R043) reacts with β - naphthylamide to form red coloured schiff base.

Positive reaction is indicated by development of cherry-red to red colour. No colour change indicates negative reaction.

Salt tolerance (1%)

Certain organisms are able to tolerate high concentration of salt. The growth medium contains sodium chloride at a concentration of 1%.

Urease Test

This test detects the ability of an organism to split urea to ammonia by the action of enzyme urease. In case of positive test, the medium turns pink under alkaline conditions due to phenol red indicator in the medium. No change in colour indicates negative reaction.

Voges-Proskauer test

Some organisms have the ability to produce a neutral end product acetyl methyl carbinol (acetoin) from glucose utilization. This can be detected by addition of 1 - 2 drops of Barritt Reagent A (R029) and 1 - 2 drops of Barrit Reagent B (R030). A positive test is indicated by pinkish red colour within 2 - 5 minutes. No change in colour indicates negative test.

Supplementary material available from HiMedia

(R008)	Kovac's Indole Reagent
(I007)	Methyl Red Indicator
(R029)	Barritt Reagent A for VP test
(RO30)	Barrit Reagent B for VP test
(R036)	TDA Reagent for Phenylalanine Deamination test
(R009)	α-Naphathylamine solution for Nitrate test
(R015)	Sulphanilic acid, 0.8% for Nitrate test
(R043)	PYR Reagent
(R026)	Gordon -McLeod Reagent(Oxidase Reagent)
(DD018)	Oxidase Discs

Disposal of used material

After use, all kits must be autoclaved or incinerated.

Storage & Shelf-life

Kit is recommended to be stored at 2-8^{0}C. Shelf-life is 12 months.

References

1. Collee J.G., Marmin B.P., Fraser A.G. and Simmons A (eds.) Mackie and McCartney Practical Medical Microbiology, (1996), 14th edition, Churchill Livingstone, New York.
2. MacFaddin, J.F. (2000) (ed.) Biochemical Tests for identification of Medical Bacteria, 3rd edition, Lippincott Williams and Wilkins, New York.
3. Murray P.R., Baron J.L., Pfaller M.A., Tenover F.C. and Yolken R.H., (eds.) (1999) Manual of Clinical Microbiology 7th edition, ASM Press, Washington, D.C.

Biochemical Identification Test Kits

HiMedia's HiSalmonella™ Identification Kit (KB011)

Control

Inoculated with *S.* serotype Enteritidis

Code	Product	Packing
KB001-10KT KB001-20KT	**HiIMViC™ Biochemical Test Kit** **a combination of 12 tests for differentiation of *Enterobacteriaceae* species.** (Kit contains sterile media for Indole, Methyl red, Voges Proskauer's, Citrate utilization tests and 8 different carbohydrates - Glucose, Adonitol, Arabinose, Lactose, Sorbitol, Mannitol, Rhamnose, Sucrose). ***Reagents supplied with kit:** Kovac's Reagent (R008) for Indole Test, Methyl Red Indicator (I007), Barritt Reagent A (R029) and Barritt Reagent B (R030) for VP Test.	10 Kits 20 Kits
KB002-10KT KB002-20KT	**HiAssorted™ Biochemical Test Kit** **a combination of 12 tests for identification of Gram-negative rods.** (Kit contains sterile media for Citrate utilization, Lysine utilization, Ornithine utilization, Urease detection, Phenylalanine deamination (TDA), Nitrate reduction, H_2S production test and 5 different carbohydrates for fermentation test -Glucose, Adonitol, Lactose, Arabinose, Sorbitol). ***Reagents supplied with kit:** TDA Reagent (R036), Nitrate Reagents : α - Naphthylamine Solution (R009) and Sulphanilic Acid 0.8% (R015).	10 Kits 20 Kits
KB003-10KT KB003-20KT	**Hi25™ Enterobacteriaceae Identification Kit** **a combination of 25 tests for identification of *Enterobacteriaceae* species.** (Kit contains discs for oxidase test and sterile media for ONPG, Lysine utilization, Ornithine utilization, Urease detection, Phenylalanine deamination (TDA), Nitrate reduction, H_2S production, Citrate utilization, Voges Proskauer's, Methyl red, Indole, Malonate, Esculin hydrolysis tests and 11 different carbohydrates utilization test - Arabinose, Xylose, Adonitol, Rhamnose, Cellobiose, Melibiose, Saccharose, Raffinose, Trehalose, Glucose, Lactose). ***Reagents supplied with kit:** TDA Reagent (R036), Nitrate Reagents : α - Naphthylamine Solution (R009) and Sulphanilic Acid 0.8% (R015), Barritt Reagent A (R029), Barritt Reagent B (R030) for VP Test, Methyl Red Indicator (I007) and Kovac's Reagent (R008) for Indole Test.	10 Kits 20 Kits
KB004-10KT KB004-20KT	**HiStaph™ Identification Kit** **a combination of 12 tests for identification of *Staphylococcus* species.** (Kit contains sterile media for Voges Proskauer's, Phosphatase, ONPG, Urease production, Arginine utilization tests and 7 different carbohydrates utilization tests - Mannitol, Sucrose, Lactose, Arabinose, Raffinose, Trehalose, Maltose). ***Reagents supplied with kit:** Barritt Reagent A (R029) and Barritt Reagent B (R030) for VP Test.	10 Kits 20 Kits
KB005-10KT KB005-20KT	**HiStrep™ Identification Kit** **a combination of 12 tests for identification of *Streptococcus* species.** (Kit contains sterile media for Voges Proskauer's, Esculin hydrolysis, PYR, ONPG, Arginine utilization tests and 7 different carbohydrates utilization tests - Glucose, Ribose, Arabinose, Sucrose, Sorbitol, Mannitol, Raffinose). ***Reagents supplied with kit:** Barritt Reagent A (R029) and Barritt Reagent B (R030) for VP Test, PYR Reagent (R043).	10 Kits 20 Kits
KB006-10KT KB006-20KT	**HiCandida™ Identification Kit** **a combination of 12 tests for identification of *Candida* species.** (Kit contains sterile media for Urease production, and 11 different carbohydrates utilization tests - Melibiose, Lactose, Maltose, Sucrose, Galactose, Cellobiose, Inositol, Xylose, Dulcitol, Raffinose, Trehalose).	10 Kits 20 Kits
KB007-10KT KB007-20KT	**HiVibrio™ Identification Kit** **a combination of 12 tests for identification of *Vibrio* species.** (Kit contains sterile media for Voges Proskauer's, Arginine utilization, Salt tolerance, ONPG, Citrate utilization, Ornithine utilization and 6 different carbohydrates utilization tests - Mannitol, Arabinose, Sucrose, Glucose, Salicin, Cellobiose). ***Reagents supplied with kit:** Barritt Reagent A (R029) and Barritt Reagent B (R030) for VP Test.	10 Kits 20 Kits
KB008-10KT KB008-20KT	**HiNeisseria™ Identification Kit** **a combination of 12 tests for identification of *Neisseria* species.** (Kit contains sterile media for Urease production, ONPG, Voges Proskauer's, Oxidase, Catalase, Nitrate reduction test and 6 different carbohydrates utilization tests - Glucose, Maltose, Lactose, Sucrose, Fructose, Mannose). ***Reagents supplied with kit:** Barritt Reagent A (R029) and Barritt Reagent B (R030) for VP Test, Gordon-McLeod Reagent (Oxidase Reagent) (R026), Nitrate Reagents : α - Naphthylamine Solution (R009) and Sulphanilic Acid 0.8% (R015).	10 Kits 20 Kits

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Result Interpretation chart

Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
Alkaline phosphatase	1-2 drops of 40% NaOH	Detects ability of organism to produce sufficient phosphatase enzyme	Cream	Pink	Cream
Amino acid utilization					
Arginine utilization	—	Detects Arginine decarboxylation	Olive green to light purple	Purple / dark purple	Yellow
Lysine utilization	—	Detects Lysine decarboxylation	Olive green to light purple	Purple / dark purple	Yellow
Ornithine utilization	—	Detects Ornithine decarboxylation	Olive green to light purple	Purple / dark purple	Yellow
Catalase	3% H_2O_2	Detects Catalase activity	Colourless	Effervescenence coming out from the loop	No effervescence seen
Citrate utilization	—	Detects capability of organism to utilize citrate as a sole carbon source	Green	Blue	Green
Glucuronidase	—	Detects Glucuronidase activity	Colourless	Bluish green	Colourless
H_2S production	—	Detects H_2S production	Orangish yellow	Black	Orangish yellow
Indole	1-2 drops of Kovac's indole reagent	Detects deamination of tryptophan	Colourless	Reddish pink	Colourless
Malonate utilization	—	Detects capability of organism to utilize sodium malonate as a sole carbon source	Light green	Blue	Light green
Methyl red	1-2 drops of Methyl red reagent	Detects acid production	Colourless	Red	Yellowish-orange
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
ONPG	—	Detects β–galactosidase activity	Colourless	Yellow	Colourless
Oxidase	—	Done on Oxidase disc separately. Detects cytochrome oxidase production.	Colourless	Deep purple	No change in colour or Purplish blue colour after 60 seconds
Phenylalanine Deamination	2-3 drops of TDA reagent	Detects Phenylalanine deamination activity	Colourless	Green	Colourless
PYR	1-2 drops of PYR Reagent	Detects PYR enzyme activity	Cream	Cherry Red	Cream
Salt tolerance (1%)	—	Detects presence of growth	Reddish purple	Growth	Reddish purple w/o growth
Urease	—	Detects Urease activity	Orangish yellow	Pink	Orangish yellow
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
Esculin hydrolysis	—	Detects esculin hydrolysis	Cream	Black	Cream
Carbohydrate utilization					
Arabinose	—	Arabinose utilization	Pinkish red / Red	Yellow	Red / Pink
Adonitol	—	Adonitol utilization	Pinkish red / Red	Yellow	Red / Pink
Cellobiose	—	Cellobiose utilization	Pinkish red / Red	Yellow	Red / Pink
Dextrose	—	Dextrose utilization	Pinkish red / Red	Yellow	Red / Pink
Dulcitol	—	Dulcitol utilization	Pinkish red / Red	Yellow	Red / Pink
Fructose	—	Fructose utilization	Pinkish red / Red	Yellow	Red / Pink
Glucosamine	—	Glucosamine utilization	Orangish Red	Yellow	Orangish Red
Glucose	—	Glucose utilization	Pinkish red / Red	Yellow	Red / Pink
Glycerol	—	Glycerol utilization	Pinkish red / Red	Yellow	Red / Pink
Galactose	—	Galactose utilization	Pinkish red / Red	Yellow	Red / Pink
Inositol	—	Inositol utilization	Pinkish red / Red	Yellow	Red / Pink
Inulin	—	Inulin utilization	Pinkish red / Red	Yellow	Red / Pink
Lactose	—	Lactose utilization	Pinkish red / Red	Yellow	Red / Pink
Maltose	—	Maltose utilization	Pinkish red / Red	Yellow	Red / Pink
Mannitol	—	Mannitol utilization	Pinkish red / Red	Yellow	Red / Pink
Melezitose	—	Melezitose utilization	Pinkish red / Red	Yellow	Red / Pink
Melibiose	—	Melibiose utilization	Pinkish red / Red	Yellow	Red / Pink
Mannose	—	Mannose utilization	Pinkish red / Red	Yellow	Red / Pink
α-Methyl-D glucoside	—	α-Methyl-D glucoside utilization	Pinkish red / Red	Yellow	Red / Pink
α-Methyl-D mannoside	—	α-Methyl-D mannoside utilization	Pinkish red / Red	Yellow	Red / Pink
Raffinose	—	Raffinose utilization	Pinkish red / Red	Yellow	Red / Pink
Ribose	—	Ribose utilization	Orangish Red	Yellow	Orangish Red
Rhamnose	—	Rhamnose utilization	Pinkish red / Red	Yellow	Red / Pink
Saccharose	—	Saccharose utilization	Pinkish red / Red	Yellow	Red / Pink
Sodium gluconate	—	Sodium gluconate utilization	Pinkish red / Red	Yellow	Red / Pink
Sucrose	—	Sucrose utilization	Pinkish red / Red	Yellow	Red / Pink
Salicin	—	Salicin utilization	Pinkish red / Red	Yellow	Red / Pink
Sorbitol	—	Sorbitol utilization	Pinkish red / Red	Yellow	Red / Pink
Trehalose	—	Trehalose utilization	Pinkish red / Red	Yellow	Red / Pink
Xylitol	—	Xylitol utilization	Pinkish red / Red	Yellow	Red / Pink
Xylose	—	Xylose utilization	Pinkish red / Red	Yellow	Red / Pink

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