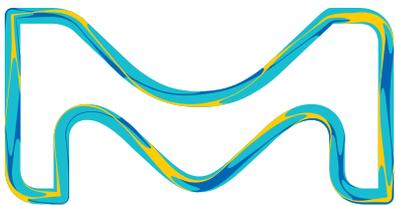


# Disks and Strips

Classical tests for microbial  
identification and confirmation



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Preparation, Separation,  
Filtration & Monitoring Products

# Classical Microbiological Tests

Disks and Strips are very helpful for the identification and confirmation of microorganisms or to monitor sterilization. They are based on rapid methods, easy to use and a great value.

The strips and disks are made of a cellulose-based material impregnated with the appropriate chemical reagents. This converts them into intelligent systems that can be used for the detection of specific abilities and properties of microorganisms, either based on the detection of enzymes by providing chromogenic substrates, or on the formation of a colored complex. The sensitivity to certain inhibitory substances can be also be tested for.



# Aminopeptidase Test (Gram-Positive Test)

## Cat. No. 75554

A test that identifies Gram-positive microorganisms by testing for the presence of L-alanine aminopeptidase

### Composition

Each package contains 50 test strips. The reaction zone of each strip contains 0.5 µmol L-alanine-4-nitroanilide and buffering agents.

### Storage

Store in a dry place at 2–8 °C. After opening the package, strips can be used until the expiry date.

### Instructions

Remove a small sample from a single colony with an inoculation loop and suspend it in 0.2 mL of distilled water in a test tube. Place a test strip into the clear opalescent suspension and incubate for between 10 and 30 minutes at 37 °C. In the presence of L-aminopeptidase, the solution becomes yellow, indicating that the microorganisms in the sample are Gram-negative (*Bacteroides vulgatus*, *Bacteroides fragilis*, *Campylobacter sp.*, *Veillonella parvula* are exceptions to this general rule.). If no yellow coloration appears, then L-alanine aminopeptidase is absent and therefore the microorganisms are Gram-positive.

Note: The growth medium from which the colonies are taken should not contain any dyes or indicators. Testing pigmented colonies is not recommended.

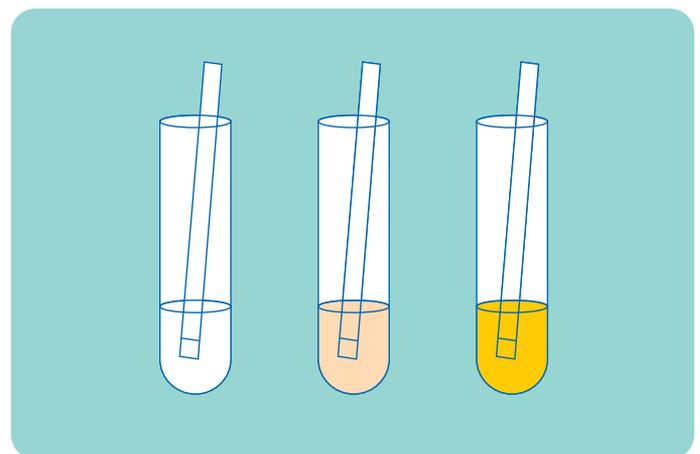
### Principle and interpretation of results

L-alanine aminopeptidase is an enzyme from the bacterial cell wall that cleaves L-alanine from various peptides, and it is found almost only in Gram-negative microorganisms. Gram-positive or Gram- variable microorganisms show no or very weak activity. The aminopeptidase test is a reliable method for determining Gram behavior. However, it cannot replace Gram-staining, as it does not detect morphology, but it is an alternative test which has a better and easier color differentiation.

| Organisms   | L-alanine aminopeptidase    |
|---|-----------------------------|
| Gram-negative bacteria<br><i>(Exceptions: Bacteroides vulgatus, Bacteroides fragilis, Campylobacter sp., Veillonella parvula)</i> | Present (yellow)            |
| Gram-positive bacteria  | Not present (no coloration) |

### References

1. G.M. Carlone, M.J. Valdez, and M.J. Pickett. Method for Distinguishing Gram-positive from Gram-negative Bacteria., J. Clinical Microbiology., 16,1157 (1982)
2. E.H. Lennette, A. Balows, W.J. Haulser, and J.P. Truant (eds.), Manual of Clinical Microbiology, 3rd edition. American Society for Microbiology. (1980)
3. J.D. Costin, M. Kappner, W. Schmidt: Differenzierung von Gram-positiven Bakterien und Gram-negativen Bakterien mit dem L-Alanin Aminopeptidase Test, Forum Mikrobiol., 351 (1983)
4. G. Cerny, Method for Distinction of the Gram-Negative from Gram-Positive Bacteria, Eur. J. Appl. Microbiol., 3, 223 (1976)
5. G. Cerny, Studies on the Aminopeptidase-Test for the Distinction of the Gram-Negative from Gram-Positive Bacteria, Eur. J. Appl. Microbiol. Biotechnol., 5, 113 (1978)
6. I. Otte, A. Tolle, Aminopetidase- und Gram-Reaktion von Bakterien, Milchwiss., 35, 215 (1980)



### Aminopeptidase Test (from left to right)

- Negative reaction
- Positive reaction
- Positive reaction

# Bacitracin Disks

## Cat. No. 08382

Disks for the presumptive identification of group A  $\beta$ -hemolytic streptococci and for differentiation between group A  $\beta$ -hemolytic streptococci and other  $\beta$ -hemolytic streptococci

The bacitracin test should be performed together with the SXT (sulfamethoxazole/ trimethoprim) susceptibility test, as their combined results increase the sensitivity and reliability of the identification result.

### Contents

Each package contains 50 sterile filter paper disks 6 mm diameter, each impregnated with 0.04 Units of bacitracin.

### Storage

Store in the freezer at or below  $-18\text{ }^{\circ}\text{C}$  in the containers provided. Allow to equilibrate to room temperature before opening. Return to freezer immediately after use.

### Instructions

Prepare blood agar (Cat. No. 70133) plates and inoculate the plates with the suspect organism using surface spreading to obtain confluent growth. Place the bacitracin disks aseptically onto the inoculated surface and press gently. Invert the plates and incubate at  $35\text{--}37\text{ }^{\circ}\text{C}$  in  $5\text{--}10\%$   $\text{CO}_2$  (microaerophilic) for  $18\text{--}24$  hours using Anaerocult™ C (Cat. No. 132383), until colony growth is observed. Check for the zone of inhibition around the disk.

### Interpretation of results

A zone of inhibition greater than or equal to 14 mm indicates sensitivity to bacitracin and is presumptive of group A streptococci. For further confirmation serological grouping is recommended.

### Quality control

The table below illustrates a range of performance control strains in routine use (blood agar plate incubated at  $35\text{--}37\text{ }^{\circ}\text{C}$  for  $18\text{--}24$  hours).

| Test organisms (ATCC®)                  | Diameter of zone of inhibition |
|---|--------------------------------|
| <i>Streptococcus pyogenes</i> (19615)   | 15 mm                          |
| <i>Streptococcus agalactiae</i> (27956) | < 14 mm                        |

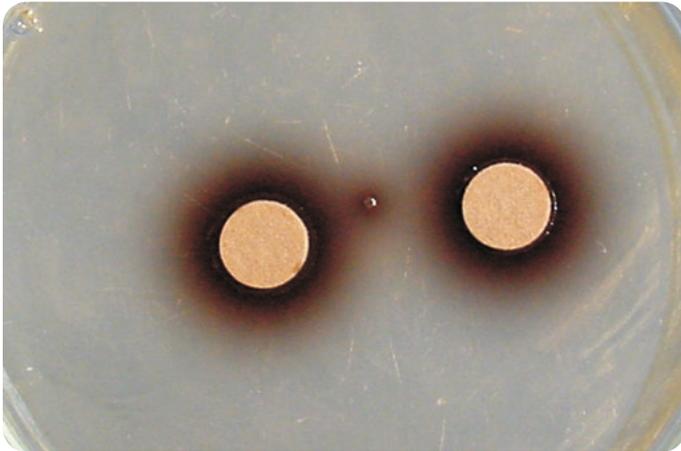
### References

1. E.M. Barnes, G.C. Mead, C.S. Impey, B.W. Adams, The Effect of Dietary Bacitracin on the Incidence of *Streptococcus faecalis* Subspecies Liquefaciens and Related Streptococci in the Intestines of Young Chicks. *Brit Poult Sci* 19: 713-723. (1978)
2. E.J. Baron, S.M. Finegold, Bailey and Scott's Diagnostic Microbiology, 8th ed. St. Louis: Mosby (1990)
3. A. Balows, W.J. Hausler, K.L. Herman, et al., Manual of Clinical microbiology, 5th ed. Washington, DC: ASM (1991)
4. MacFaddin J.F., Biochemical Tests for the Identification of Medical Bacteria., 3rd ed. Philadelphia: Lippincott Williams & Wilkins (2000)

# Bile Esculin Disks

## Cat. No. 80507

Disks used for rapid detection of esculin hydrolysis in the presence of bile



The detection of esculin hydrolysis in the presence of bile allows group D streptococci to be differentiated from non-group D streptococci, as only group D streptococci can hydrolyze the esculin to esculetin and dextrose. The resulting esculetin then reacts with iron salts such as ferric citrate, forming a blackish-brown colored complex.

### Contents

Each package contains 50 sterile filter paper disks of 6 mm diameter, each impregnated with esculin.

### Instructions

Place a bile esculin disk on the seeded bile esculin agar base plate (containing no esculin) or another media. Incubate at 35 °C for 18–24 hours.

### Quality control

Culture characteristics after 18–24 hours at 35 °C.

| Test organisms (ATCC®)                | Esculin hydrolysis |
|---------------------------------------|--------------------|
| <i>Streptococcus faecalis</i> (29212) | +                  |
| <i>Streptococcus pyogenes</i> (19615) | -                  |
| <i>Listeria monocytogenes</i> (19118) | +                  |

### References

1. Rochaix, C.R.Soc. Biol., 90, 771 (1924)
2. Meyer and Schönfeld, Zentralbl. Bacteriol. Parasitenkd. Infektionskr. Hyg. Abt. I Orig., 99, 402 (1924)
3. J.F. MacFaddin, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore (1980)
4. A.E. Greenberg, R. R. Trussell and L. S. Clesceri (Eds.), Standard Methods for the Examination of Water and Wastewater, 16th ed., A.P.H.A., Washington D.C. (1985)
5. R.R.Facklam, M.D. Moody, Presumptive Identification of Group D Streptococci: the Bile-Esculin Test. Appl. Microbiol., 20, 245 (1970)
6. S.C. Edberg, S. Pittman, J.M. Singer, Esculin Hydrolysis by Enterobacteriaceae., J. Clin. Micro. 6, 111 (1977)

# Carbohydrates Differentiation Discs

## Differentiate bacteria based on their fermentation profile

The table below shows for which carbohydrates the differentiation discs are available.

Any liquid, semisolid, or solid media can be used. Liquid and semisolid media are dispensed in 5 ml aliquots into test tubes to observe for fermentation activity. A single carbohydrate disc inoculated with the test organism is added to each tube.

| Cat. # | Carbohydrate | Code on discs |
|--------|--------------|---------------|
| 55876  | Adonitol     | (Ad)          |
| 80372  | Arabinose    | (Ar)          |
| 56481  | Cellobiose   | (Ge)          |
| 63367  | Dextrose     | (De)          |
| 73044  | Dulcitol     | (Du)          |
| 53901  | Fructose     | (Fc)          |
| 89608  | Galactose    | (Ga)          |
| 89614  | Inositol     | (Is)          |
| 90058  | Inulin       | (In)          |
| 28816  | Lactose      | (La)          |
| 77653  | Maltose      | (Ma)          |
| 94438  | Mannitol     | (Mn)          |
| 94445  | Mannose      | (Mo)          |
| 93196  | Melibiose    | (Mb)          |
| 94226  | Raffinose    | (Rf)          |
| 93999  | Rhamnose     | (Rh)          |
| 92971  | Salicin      | (Sa)          |
| 93998  | Sorbitol     | (Sb)          |
| 94309  | Sucrose      | (Su)          |
| 92961  | Trehalose    | (Te)          |
| 07411  | Xylose       | (Xy)          |

Store disks in tightly sealed vials in a dry, light-protected place at the recommended temperature (see product label).

### Contents:

One vial contains 25 sterile filter paper discs impregnated with the respective carbohydrate.



Carbohydrate Discs

### Instructions:

A sugar-free medium base of choice is prepared, then dispensed and sterilized. The following media are recommended for this test:

#### Liquid media

Andrade Peptone Water (Cat. No. A0715)  
Andrade Peptone Water with Meat Extract  
Phenol Red Broth Base (Cat. No. P8976)  
Phenol Red Broth with Meat Extract  
Purple Broth  
Yeast Fermentation Broth

#### Semisolid media

Cystine Tryptone Agar  
OF Basal Medium (Cat. No. 75315)  
Tryptone Agar

#### Solid media

Phenol Red Agar  
Purple Agar Base  
Sanders Agar

When using semisolid media, the disc and inoculum are pushed into the medium to just beneath the surface so that the medium at the bottom of the dish can serve as a control while fermentation can be detected at the surface level. When using solid media, it is possible to detect fermentation of a number of sugars on the same plate. Sterile plates containing the agar medium of choice are surface-seeded with test organism(s), and the carbohydrate discs are pressed gently into the medium's surface at a minimum distance of 2 cm apart. Incubation is carried out at 36 °C ( $\pm 1.0$  °C) for 18–48 hours. Results are recorded once after 18–24 hours and again at 48 hours. Test plates should be checked frequently, as reversal of fermentation can take place. When using liquid medium, any gas produced during fermentation must be collected in an inverted Durham's tube. Any acid(s) produced will change the color of the medium. With semisolid media, any gas produced is trapped and observable as bubbles.

On agar plates, fermentation is indicated by a change in color around the disc.

Microorganisms can only ferment certain carbohydrates. This depends on the enzymes they possess for cleaving carbohydrates. Fermentation can be detected by gas production (CO<sub>2</sub>) in liquid media and/or color change of the pH indicator caused by acid production.

Culture characteristics when incubated in Phenol Red Broth Base, after 18–24 hours§ at 35–37 °C.

| Organisms (ATCC®)                      | Growth    | Adonitol |     | Arabinose |     | Cellobiose |     | Dextrose |     | Dulcitol |     | Fructose |     | Galactose |     |
|--|-----------|----------|-----|-----------|-----|------------|-----|----------|-----|----------|-----|----------|-----|-----------|-----|
|  |           | Acid     | Gas | Acid      | Gas | Acid       | Gas | Acid     | Gas | Acid     | Gas | Acid     | Gas | Acid      | Gas |
| <i>Citrobacter freundii</i> (8090)     | luxuriant | -        | -   | +         | +   | +          | -   | +        | +   | -        | -   | NA       | NA  | +         | +   |
| <i>Enterobacter aerogenes</i> (13048)  | luxuriant | +        | +   | +         | +   | +          | +   | +        | +   | -        | -   | +        | +   | +         | +   |
| <i>Escherichia coli</i> (25922)        | luxuriant | -        | -   | +         | +   | -          | -   | +        | +   | -        | -   | +        | +   | +         | +   |
| <i>Klebsiella pneumoniae</i> (13883)   | luxuriant | +        | +   | +         | +   | +          | +   | +        | +   | -        | -   | +        | +   | +         | +   |
| <i>Neisseria meningitis</i> (13090)    | luxuriant | NA       | NA  | NA        | NA  | NA         | NA  | +        | -   | NA       | NA  | -        | -   | -         | -   |
| <i>Proteus vulgaris</i> (13315)        | luxuriant | -        | -   | -         | -   | -          | -   | +        | +   | -        | -   | -        | -   | +         | +   |
| <i>Salmonella Typhimurium</i> (14028)  | luxuriant | -        | -   | +         | +   | -          | -   | +        | +   | +        | +   | +        | +   | +         | +   |
| <i>Salmonella Typhi</i> (6539)         | luxuriant | -        | -   | -         | -   | -          | -   | +        | -   | -        | -   | -        | -   | +         | -   |
| <i>Serratia marcescens</i> (8100)      | luxuriant | -        | -   | -         | -   | -          | -   | +        | +   | -        | -   | +        | -   | +         | -   |
| <i>Shigella flexneri</i> (12022)       | luxuriant | -        | -   | -         | -   | -          | -   | +        | -   | -        | -   | +        | +   | +         | -   |
| <i>Streptococcus pneumoniae</i> (6303) | luxuriant | NA       | NA  | +         | -   | NA         | NA  | NA       | NA  | NA       | NA  | +        | +   | NA        | NA  |
| <i>Streptococcus pyogenes</i> (19615)  | luxuriant | NA       | NA  | NA        | NA  | NA         | NA  | +        | -   | NA       | NA  | NA       | NA  | NA        | NA  |

| Organisms (ATCC®)                      | Growth    | Inositol |     | Inulin |     | Lactose |     | Maltose |     | Mannitol |     | Mannose |     | Melibiose |     |
|--|-----------|----------|-----|--------|-----|---------|-----|---------|-----|----------|-----|---------|-----|-----------|-----|
|  |           | Acid     | Gas | Acid   | Gas | Acid    | Gas | Acid    | Gas | Acid     | Gas | Acid    | Gas | Acid      | Gas |
| <i>Citrobacter freundii</i> (8090)     | luxuriant | -        | -   | NA     | NA  | +       | +   | +       | +   | +        | +   | +       | +   | -         | -   |
| <i>Enterobacter aerogenes</i> (13048)  | luxuriant | +        | +   | NA     | NA  | +       | +   | +       | +   | +        | +   | +       | +   | +         | +   |
| <i>Escherichia coli</i> (25922)        | luxuriant | -        | -   | NA     | NA  | +       | +   | +       | +   | +        | +   | +       | +   | +         | +   |
| <i>Klebsiella pneumoniae</i> (13883)   | luxuriant | +        | +   | NA     | NA  | +       | +   | +       | +   | +        | +   | +       | +   | +         | +   |
| <i>Neisseria meningitis</i> (13090)    | luxuriant | NA       | NA  | NA     | NA  | NA      | NA  | +       | -   | NA       | NA  | NA      | NA  | NA        | NA  |
| <i>Proteus vulgaris</i> (13315)        | luxuriant | -        | -   | NA     | NA  | -       | -   | +       | +   | -        | -   | -       | -   | -         | -   |
| <i>Salmonella Typhimurium</i> (14028)  | luxuriant | +        | +   | NA     | NA  | -       | -   | +       | +   | +        | +   | -       | -   | +         | +   |
| <i>Salmonella Typhi</i> (6539)         | luxuriant | -        | -   | NA     | NA  | -       | -   | +       | -   | +        | -   | +       | +   | +         | +   |
| <i>Serratia marcescens</i> (8100)      | luxuriant | +        | -   | NA     | NA  | -       | -   | +       | -   | +        | -   | +       | +   | -         | -   |
| <i>Shigella flexneri</i> (12022)       | luxuriant | -        | -   | NA     | NA  | -       | -   | +       | -   | +        | -   | +       | +   | -         | -   |
| <i>Streptococcus pneumoniae</i> (6303) | luxuriant | NA       | NA  | +      | -   | NA      | NA  | +       | -   | -        | -   | NA      | NA  | NA        | NA  |
| <i>Streptococcus pyogenes</i> (19615)  | luxuriant | NA       | NA  | -      | -   | NA      | NA  | +       | -   | NA       | NA  | NA      | NA  | NA        | NA  |

| Organisms (ATCC®)                      | Growth    | Raffinose |     | Rhamnose |     | Salicin |     | Sorbitol |     | Sucrose |     | Trehalose |     | Xylose |     |
|--|-----------|-----------|-----|----------|-----|---------|-----|----------|-----|---------|-----|-----------|-----|--------|-----|
|  |           | Acid      | Gas | Acid     | Gas | Acid    | Gas | Acid     | Gas | Acid    | Gas | Acid      | Gas | Acid   | Gas |
| <i>Citrobacter freundii</i> (8090)     | luxuriant | -         | -   | +        | +   | -       | -   | +        | +   | +       | +   | +         | +   | +      | +   |
| <i>Enterobacter aerogenes</i> (13048)  | luxuriant | +         | +   | +        | +   | +       | +   | +        | +   | +       | +   | +         | +   | +      | +   |
| <i>Escherichia coli</i> (25922)        | luxuriant | -         | -   | +        | +   | -       | -   | +        | +   | -       | -   | +         | +   | +      | +   |
| <i>Klebsiella pneumoniae</i> (13883)   | luxuriant | +         | +   | +        | +   | +       | +   | +        | +   | +       | +   | +         | +   | +      | +   |
| <i>Neisseria meningitis</i> (13090)    | luxuriant | NA        | NA  | NA       | NA  | NA      | NA  | NA       | NA  | -       | -   | NA        | NA  | NA     | NA  |
| <i>Proteus vulgaris</i> (13315)        | luxuriant | -         | -   | -        | -   | +       | +   | -        | -   | +       | +   | +         | +   | +      | [+] |
| <i>Salmonella Typhimurium</i> (14028)  | luxuriant | -         | -   | +        | +   | -       | -   | +        | +   | -       | -   | +         | +   | +      | +   |
| <i>Salmonella Typhi</i> (6539)         | luxuriant | -         | -   | -        | -   | -       | -   | +        | -   | -       | -   | +         | -   | +      | -   |
| <i>Serratia marcescens</i> (8100)      | luxuriant | -         | -   | -        | -   | +       | [+] | +        | -   | +       | +   | +         | [+] | -      | -   |
| <i>Shigella flexneri</i> (12022)       | luxuriant | -         | -   | -        | -   | -       | -   | +        | -   | -       | -   | +         | -   | -      | -   |
| <i>Streptococcus pneumoniae</i> (6303) | luxuriant | NA        | NA  | NA       | NA  | NA      | NA  | -        | -   | NA      | NA  | NA        | NA  | NA     | NA  |
| <i>Streptococcus pyogenes</i> (19615)  | luxuriant | NA        | NA  | NA       | NA  | +       | -   | NA       | NA  | +       | -   | NA        | NA  | NA     | NA  |

**Key:**

(§) longer if necessary

+ positive reaction, color changes to yellow

- negative reaction, no color change or remaining red

[+] weak/slight reaction

NA = not applicable

\* for more details, see References

\*\* when basal medium is inoculated with test organisms, there is a negative reaction, i.e., no change in color

**References:**

- Garrity, G. M.; Boone, D. R.; Castenholz, R. W., Eds. *Bergey's Manual of Systematic Bacteriology*, Vol. 1, Williams and Wilkins: Baltimore, 1984,
- Holt, J. G., et al., Eds. *Bergey's Manual of Systematic Bacteriology*, 9th ed., Williams and Wilkins: Baltimore, 1994.

# Coagulase Test (Slide)

## Cat. No. 75832

The enzyme coagulase, produced by a few *Staphylococcus* species, is a key feature of pathogenic staphylococci.

These disks detect coagulase-negative or coagulase-positive organisms (*Staphylococcus aureus*).

### Contents:

1 box contains 30 discs (made from rabbit plasma)  
Store below 2–8 °C and use before expiry date.

### Instructions:

Place one drop of distilled water on a glass microscope slide and prepare a heavy suspension of the organism to be tested. Add one Coagulase Disc and rub the disc about in the suspension, using the tip of a wire loop. Then add a second drop of distilled water, and mix again.

Coagulase-negative organisms will remain evenly suspended. Coagulase-positive organisms will cause macroscopic clumping within 30 seconds; do not observe beyond one minute as drying may give a misleading appearance of granularity. Positive and negative control cultures must be read with each test.

### Principle and interpretation:

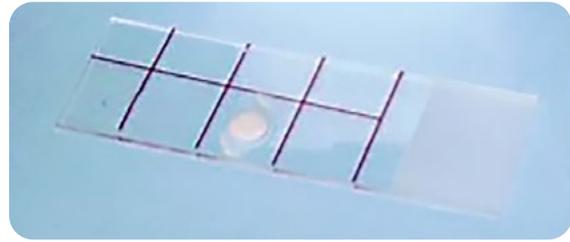
This test is to differentiate potentially pathogenic *Staphylococcus* species from other Gram-positive, catalase-positive cocci by detecting coagulase.

It is thought that an infective organism that produces the coagulase enzyme may protect itself by inducing clotting in surrounding tissue, thereby inhibiting destruction by the body's defenses, e.g. by phagocytosis or antibodies. Formation of coagulase and of enteric toxins by *Staphylococcus aureus* are very closely related. Therefore, together with the DNase test, the coagulase test is an important indicator for the pathogenicity of *Staphylococcus* strains.

*Staphylococcus aureus* produces free and bound coagulase. Free coagulase is an extracellular enzyme which reacts with prothrombin and its derivatives. Bound coagulase is localized on the surface of the cell wall and reacts with the a and b chains of the plasma fibrinogens to form a coagulate. This test detects only the bound form of coagulase.

### References:

1. W.H. Sperber, S.R. Tatini, Interpretation on the tube coagulase test for the identification of *Staphylococcus aureus*. - Appl. Microbiol., 29, 502 (1975)



Coagulase Test Disc Strip

# DMACA Indole Disks (Indole Detection Disks)

## Cat. No. 05686

Disks to determine the ability of an organism to split tryptophan into indole and  $\alpha$ -aminopropionic acid

The DMACA indole disks are used to determine the ability of an organism to split tryptophan into indole and  $\alpha$ -aminopropionic acid. The presence of indole can be detected by adding DMACA, which gives rise to a blueish-purple complex. This method makes it possible to differentiate *Escherichia coli* from *Klebsiella*.

### Contents

Each package contains 50 disks impregnated with p-dimethyl- aminocinnamaldehyde (DMACA).

### Quality control

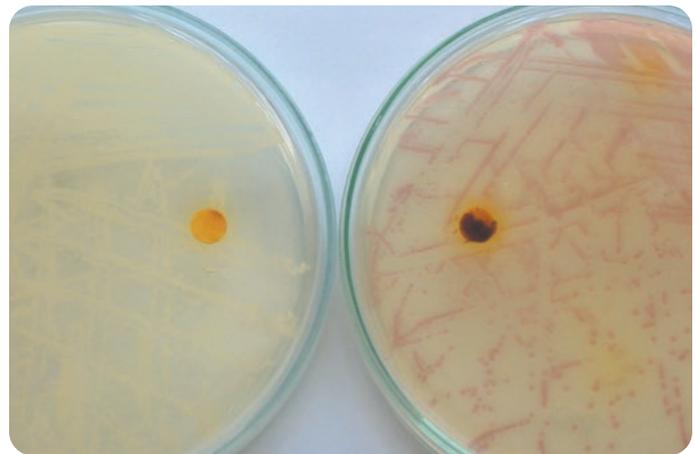
| Test organisms (ATCC®)                | DMACA |
|---------------------------------------|-------|
| <i>Escherichia coli</i> (25922)       | +     |
| <i>Pseudomonas aeruginosa</i> (27853) | -     |
| <i>Klebsiella pneumoniae</i> (13883)  | -     |

### Instructions

Place the disk on a suspect colony grown on a medium such as modified UTI ChromoSelect agar (Cat. No. 16636) or Christensen's urea agar (Cat. No. 27048). Lay the Petri dish on a white background and observe for the appearance of blue to purple color within 10–30 seconds.

### References

1. R. Vracko, J.C. Sherris, Indole-spot test in bacteriology., Am. J. Clin. Pathol., 39, 429 (1963)
2. V.L. Sutter, W.T. Carter, Evaluation of Media and Reagents for Indole-Spot Test in Anaerobic Bacteriology., Am. J. Clin. Pathol., 58, 335 (1972)
3. G.D. Fay, A.L. Barry, Methods for Detecting Indole Production by Gram-negative Non-Spore Forming Anaerobes., Appl. Micro. 27, 562 (1974)
4. D.F. Welch, P.A. Ahlin, J.M. Matsen, Differentiation of *Haemophilus spp.* in Respiratory Isolate Cultures by an Indole Spot Test., J. Clin. Micro. 15, 216 (1982)
5. H.D. Isenberg, Ed., Clinical Microbiology Procedures Handbook, Vol 1., Washington, DC, ASM (1992)
6. B.A. Forbes, D.F. Sahm, A.S. Weissfeld, Bailey and Scott's Diagnostic Microbiology., 10th ed., St Louis, Mosby (1998)
7. J.F. MacFaddin, Biochemical Tests for Identification of Medical Bacteria., 3rd ed., Philadelphia, Lippincott Williams & Wilkins (2000)



### DMACA Indole Disks

1. *Staphylococcus aureus* (left)
2. *Escherichia coli* (right)

# Hippurate Disks

## Cat. No. 40405

### Disks recommended for the qualitative detection of organisms with hippurate hydrolase activity

The hippurate disks are recommended for the qualitative detection of organisms that have the enzyme hippurate hydrolase. Hippurate hydrolase causes the hydrolysis of peptide bonds in the hippurate molecule, releasing glycine and benzoic acid as end products. The benzoic acid can be detected by using a ferric chloride indicator, and it is also possible to detect glycine with ninhydrin. However, it must be taken into account that any free amino acids will generate false positive results. The disk method is a rapid test for the presumptive identification of *Gardnerella vaginalis*, *Campylobacter jejuni*, *Listeria monocytogenes* and  $\beta$ -hemolytic group B streptococci.

### Contents

Each package contains 25 sterile filter paper disks of 10 mm diameter, each impregnated with sodium hippurate.

### Instructions

Aseptically place the hippurate disk into brain heart infusion broth (Cat. No. 53286) inoculated with a suspect colony, incubate at 35 °C for 48 hours, then separate the supernatant from the cells by centrifugation. Add 2 mL of ferric chloride reagent to 2 mL of supernatant. Shake well and check for the formation of a precipitate. If brown flocculent precipitate persists after shaking for 10 minutes, hippurate hydrolysis can be inferred.

### Preparation of ferric chloride reagent

12 g ferric chloride, 94.6 mL distilled water and 5.4 mL concentrated hydrochloric acid are needed. Pour 75 mL of distilled water in a 100 mL graduated flask. Cautiously pipette 5.4 mL of HCl into the flask and add 12 g of ferric chloride. Dissolve by warming the flask gently, swirling the contents to mix well. Bring the volume up to 100 mL with distilled water. This solution should have an orange color.

### Quality control

The table below illustrates the performance of strains used routinely for control.

| Test organisms (ATCC®)                 | Growth | Hippurate hydrolysis |
|--|--------|----------------------|
| <i>Enterococcus faecalis</i> (29212)   | +++    | -                    |
| <i>Streptococcus agalactiae</i> (4768) | +++    | +                    |
| <i>Streptococcus pyogenes</i> (19615)  | +++    | -                    |

+ = Brown flocculent precipitate persists after shaking for 10 minutes

- = In case of any visible precipitate, this can be dissolved by shaking.

### References

1. S.H. Ayers, P. Rupp, Differentiation of Hemolytic Streptococci from Human and Bovine Sources by the Hydrolysis of Sodium Hippurate. *J. Infect. Dis.*, 30, 388 (1922)
2. R.R. Facklam, et al., Presumptive identification of group A, B, and D Streptococci., *Appl. Microbiol.*, 27(1), 107 (1974)
3. S.M. Harvy, Hippurate Hydrolysis by *Campylobacter fetus.*, *J. Clin. Microbiol.* 11,435 (1980)
4. M. Hwang, G.M. Ederer, Rapid Hippurate Hydrolysis Method for Presumptive Identification of Group B Streptococci., *J. Clin. Microbiol.* 1, 114 (1975)
5. N.W. Luechtefeld, W.L. Wang, Hippurate hydrolysis by and triphenyltrazolium tolerance of *Campylobacter fetus.*, *J. Clin. Microbiol.*, 15, 137 (1982)
6. P. Piot, et al., Identification of *Gardnerella (Haemophilus) vaginalis.*, *J. Clin. Microbiol.*, 19 (1982)
7. A.E. Greenberg, R.R. Trussell, L.S. Clesceri, Eds., *Standard Methods for the Examination of Water and Wastewater*, 16th ed., APHA, Washington, DC (1985)
8. S.M. Finegold, E.J. Baron, *Bailey & Scott's Diagnostic Microbiology*, 8th Ed., St. Louis, MO, C.V. Mosby Co (1990)
9. H.D. Isenberg, Ed., *Clinical Microbiology Procedures Handbook*, Vol I & II, Washington, DC, ASM (1992)
10. P.R. Murray, et al., *Manual of Clinical Microbiology*, 6th ed., American Society for Microbiology, Washington D.C. (1995)
11. B.A. Forbes, D.F. Sahm, A.S. Weissfeld, *Bailey and Scott's Diagnostic Microbiology.*, 10th ed., St Louis, Mosby (1998)
12. E.W. Koneman, S.D. Allen, W.M. Janda, P.C. Schreckenberger, W.C. Winn, Eds., *Color Atlas and Textbook of Diagnostic Microbiology*, 5th ed., Philadelphia: Lippincott Williams & Wilkins (1997)
13. Mackie and McCartney, *Practical Medical Microbiology* 14th ed., Vol. 2, Collee, Duguid, Fraser and Marmion, Eds., Churchill Livingstone, Edinburgh (2000)

# Hippurate Strips Kit

## Cat. No. 01869

Kit recommended to detect organisms that have hippurate hydrolase

The strips detect hippurate hydrolase activity of group *B streptococci*, *Campylobacter jejuni*, *Gardnerella vaginalis*, and other microorganisms.

The test is based on the hydrolysis of substrate (sodium hippurate) by the bacterial enzyme hippurate hydrolase, and the production of benzoic acid and glycine. Glycine produced by this enzymatic reaction is detected after 24 hours of incubation in a reaction with a chromogen (ninhydrine) and is indicated by the presence of a blue-purple color.

### Contents

The kit contains 50 hippurate strips (Cat. No. 92472) saturated with sodium hippurate and 350 mg chromogen in 15 mL of diluent (Cat. No. 01875)

### Storage

Store in dry location at temperature between 2 and 8 °C. See product label for expiration date.

### Instructions

1. Prepare bacterial suspension from pure 24 hour culture of tested microorganisms in 0.5 mL of saline solution. (Use a narrow tube so that the entire active zone of the strip can be exposed to the suspension. The density of the suspension can reach up to 2 ° on the McFarland turbidity scale).
2. Dip substrate strip into tube with suspension so that the entire active zone is submerged; mix gently.
3. Incubate at 37 °C for 24 hours.
4. When incubation is complete, drip approximately 200 µL (4 drops) of reagent onto the inner wall of the tube. Do not mix.
5. Incubate at laboratory temperature (18–24 °C) for 5–10 minutes.
6. Evaluate result of test.

### Interpretation of results

**Negative reaction:** no color change occurs

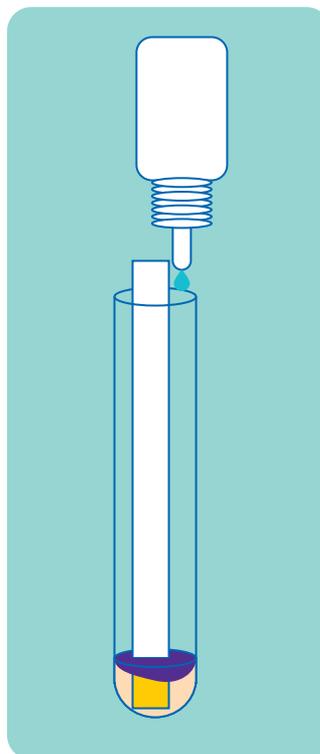
**Positive reaction:** a blue to purple color “ring” develops where the reagent and inoculum come into contact

The table below lists control strains in routine use:

| Test organisms (ATCC®)                 | Result   |
|--|----------|
| <i>Enterococcus faecalis</i> (29212)   | negative |
| <i>Streptococcus agalactiae</i> (4768) | positive |
| <i>Bacillus subtilis</i> (31193)       | positive |
| <i>Streptococcus pyogenes</i> (19615)  | negative |

### References

1. Barrow, G. I.; Feltham, R. K. A., Eds. *Cowan and Steels: Manual for the Identification of Medical Bacteria*, Cambridge University: Cambridge, U.K., 1993, pp 220, 221, 229.



# Hydrogen Sulfide Test Strips (Lead Acetate Test Strips, H<sub>2</sub>S Test Strips)

Cat. No. 06728

Test strips for the detection of H<sub>2</sub>S production by microorganisms

A large number of bacteria can produce H<sub>2</sub>S in small amounts from sulfur-containing amino acids in carbohydrate media. In the presence of lead acetate, the H<sub>2</sub>S will produce a black precipitate, giving rise to a visible black color on the paper strip. The lead acetate method is very sensitive, allowing the detection of trace levels of hydrogen sulfide.

## Contents

Each package contains 25 sterile filter paper strips impregnated with lead acetate.

## Instructions

Inoculate peptone water (Cat. No. 70179) with the suspect organism. Insert a lead acetate paper strip between the plug and inner wall of tube, above the inoculated medium, and incubate at 35 °C for 18–24 hours.

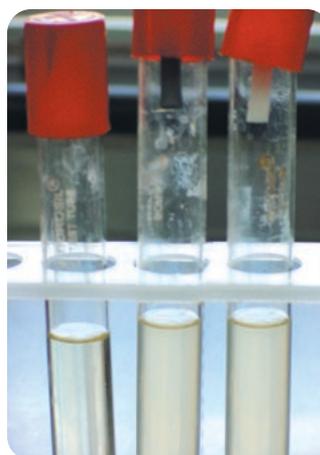
## Quality control

Culture response after 18–24 hours at 35 °C: a positive reaction appears as a blackening of the lower part of the strip. No blackening should appear if the result is negative.

| Test organisms (ATCC®)                | H <sub>2</sub> S production |
|---------------------------------------|-----------------------------|
| <i>Escherichia coli</i> (25922)       | -                           |
| <i>Salmonella Enteritidis</i> (13076) | +                           |
| <i>Salmonella Typhimurium</i> (14028) | +                           |

## References

1. R.N. Collins, M.D. Treger, J.B. Goldsby, J.R. III Boring, D.B. Coohon, R.N. Barr. Interstate outbreak of *Salmonella newbrunswick* infection traced to powdered milk., *JAMA*, 203, 838 (1968)
2. J.B. Weissman, R.M.A.D. Deen, M. Williams, N. Swanston, S. Ali, An island-wide epidemic of salmonellosis in Trinidad traced to contaminated powdered milk., *West Indian Med. J.*, 26, 135 (1977)
3. S.M. Finegold, W.J. Martin, Bailey and Scott's Diagnostic Microbiology 6th ed., The CV. Mosby Co., St. Louis (1982)
4. J.F. MacFaddin, Media For Isolation-Cultivation-Identification-Maintenance of Medical Bacteria., Vol. 1, Williams and Wilkins, Baltimore (1985)
5. B. Rowe, N.T. Begg, D.N. Hutchinson, et al., Salmonella Ealing Infections Associated with Consumption of Infant Dried Milk., *Lancet*, 2, 900 (1987)
6. M.D. Sobsey, F.K. Pfaender, Evaluation of the H<sub>2</sub>S Method for Detection of Fecal Contamination of Drinking Water, WHO (2008)



**Image: Hydrogen Sulfide Test Strips (from left to right)**

1. Control
2. *Salmonella* serotype Typhimurium
3. *Escherichia coli*

# Indoxyl Strips (Acetoxyindol Strips)

## Cat. No. 04739

### A test for the rapid detection of microbial acetate esterase activity

The test is based on the reaction of free indoxyl groups with oxygen, which results in a color change. The enzyme acetate esterase is present in species belonging to *Campylobacter* and in *Branhamella catarrhalis*. The test is suitable for screening examinations and for the identification of suspect colonies.

#### Contents

Each package contains 100 plastic test strips with an active zone saturated with 3-acetoxy indol, the substrate for acetate esterase.

#### Storage

Store dry at +2 to +8 °C. Shelf life can be extended by storing the product at -20 °C.

#### Instructions

Wipe off several the suspect colonies that have grown for 18–24 hours in a Petri dish, using the active zone of diagnostic strip. The result can be read after 3–5 minutes. For accurate results, store dry. The aluminum tube containing the strips should not be opened before the temperature is equilibrated in order to prevent condensation forming on the strips. For good test performance it is required that there is sufficient humidity on the cultures or the cultivation media where the suspect colonies are tested. If there is insufficient humidity, the active zone of the strip can be moisturized by using condensed water from the lid of the dish or by adding approximately 10 µL of distilled water. To wipe the colonies, do not use a microbiological loop made of metal.

#### Interpretation of results

##### Negative reaction

No color change develops at the position of a colony on the strip.

##### Positive reaction

A blue-green spot develops at the position of a colony on the strip.



Indoxyl Strips

#### Quality control

The table above illustrates the performance of strains used routinely for control.

#### References

1. F.J. Bolton, D.R.A. Wareing, et al., Identification and Biotyping of Campylobacters. 29, 151. In: R.G. Board, D. Jones and F.A. Skinner. Identification Methods in Applied and Environmental Microbiology. Academic Press: London (1992)
2. J. Klena, A Survey of Phenotypic and Genetic Methods Used to Identify and Differentiate Thermotolerant Campylobacter spp. Strains, report of Ministry of Health Department of Plant and Microbial Sciences University of Canterbury (2001)

| Test organisms (ATCC®)                 | Result   |
|--|----------|
| <i>Escherichia coli</i> (25922)        | negative |
| <i>Branhamella catarrhalis</i> (25238) | positive |

# Kovac's Reagent Strips (Indole Reagent Test strips according to Kovac)

Cat. No. 78719

Strips to determine the ability of microorganisms, primarily *Enterobacteriaceae*, to use tryptophanase.

The enzyme tryptophanase, present in species such as *Escherichia coli*, cleaves tryptophan to indole and  $\alpha$ -aminopropionic acid. The p-amino-benzaldehyde present in the reagent reacts with the indole to form a pink complex.

## Contents

Each package contains 25 sterile filter paper strips impregnated with Kovac's reagent. The Kovac's reagent is prepared by dissolving 10 g of p-aminobenzaldehyde in 150 mL of isoamylalcohol, then slowly adding 50 mL of concentrated hydrochloric acid.

## Instructions

Indole production by a positive organism is observed after inserting a Kovac's reagent strip between the plug and inner wall of the tube, above the inoculated peptone water (Cat. No. 70179), and incubation at 35 °C for 18–24 hours.

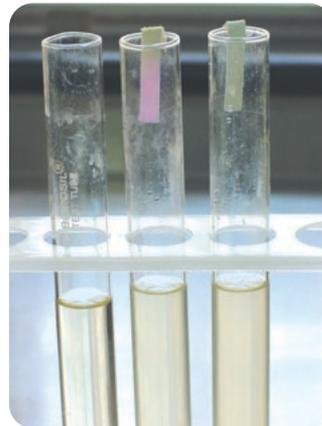
## Quality control

Culture response appears after incubation in peptone water (Cat. No. 70179) for 18–24 hours at 35 °C. Pink coloration on the lower part of the strip should be considered a positive reaction, indicating the microorganisms have tryptophanase activity. If there is no color change the test result is negative.

| Test organisms (ATCC®)                | Indole production |
|---------------------------------------|-------------------|
| <i>Escherichia coli</i> (25922)       | +                 |
| <i>Enterobacter aerogenes</i> (13048) | -                 |

## References

1. J.F. MacFaddin, *Biochemical Tests for Identification of Medical Bacteria*, 2nd ed., Williams and Wilkins, Baltimore (1980)
2. United States Environmental Protection Agency, Office of Water (4303T), Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC), Washington, DC (2014)



## Kovac's Reagent Strips (from left to right)

Control

*Escherichia coli*

*Staphylococcus aureus*

# Nitrate Reagent Disks Kit

## Cat No. 51138

### Disks to detect the reduction of nitrate

The nitrate test detects activity of the enzyme nitrate reductase, which reduces nitrate to nitrite in the presence of a suitable electron donor. Nitrite can be detected by using an appropriate colorimetric reagent.

#### Contents

Each package contains 50 sterile filter paper discs (diameter 6 mm) impregnated with nitrate reagent (Cat. No. 08086) and a vial containing 5 mL of rehydrating fluid (Cat. No. 43505).

#### Storage

Store in the freezer below 4 °C in the containers provided with the product. Allow to equilibrate to room temperature before opening. Return to freezer storage immediately after use.

#### Instructions

The test culture should be grown on a suitable agar medium plate containing the nitrate substrate. Place the nitrate reagent disks on a suspected colony. For enhanced color intensity add a drop of the rehydrating fluid provided in the kit.

#### Principle

Reduction of nitrate ( $\text{NO}_3$ ) to nitrite ( $\text{NO}_2$ ) and to nitrogen gas ( $\text{N}_2$ ) usually takes place under anaerobic conditions<sup>1</sup>. Most facultative anaerobes can reduce nitrate in the absence of oxygen and almost all Enterobacteriaceae are able to reduce nitrate. This anaerobic respiration is an oxidation process in which inorganic substances provide oxygen to serve as an electron acceptor and provide energy<sup>2</sup>. Depending on the bacterial species, the nitrate reduction results in the production of various end products, the most common of which is molecular nitrogen by way of nitrite reduction<sup>2</sup>. Depending upon the environmental conditions, these products are usually not further oxidized or assimilated into the cellular metabolism, but are excreted into the surrounding medium.

#### Interpretation of results

Color change of the disk to red-pink indicates positive nitrate reduction reaction.

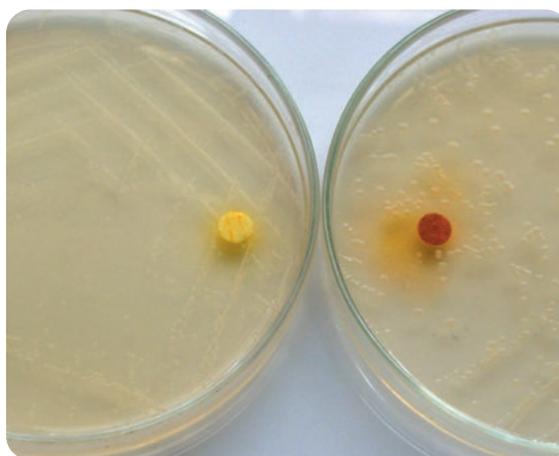
#### Quality control

The table below illustrates the performance of strains used routinely for control.

| Test organisms (ATCC®)                     | Growth | Nitrate reduction |
|--|--------|-------------------|
| <i>Acinetobacter calcoaceticus</i> (19606) | +++    | -                 |
| <i>Enterobacter aerogenes</i> (13048)      | +++    | +                 |
| <i>Escherichia coli</i> (25922)            | +++    | +                 |
| <i>Salmonella Typhimurium</i> (14028)      | +++    | +                 |

#### References

1. Jr. M.J. Pelczar, R.D. Reid, Microbiology, 2nd ed., McGraw-Hill, New York, 567 (1965)
2. R.Y. Stainer, M. Douderoff, E.A. Adelberg, The Microbial World, 2nd ed., Prentice-Hall, 116-117 (1963)
3. Wideman P.A., Citronbaum D.M., Sutter V.L., Simple Disk Technique for Detection of Nitrate Reduction in Anaerobic Bacteria, J Clin Micro, 5(3):315-9 (1977)
4. Lennette E.H., Balows A., Hausler W.J., et al., Manual of Clinical Microbiology, 4th ed. Washington, DC: ASM (1985)
5. Baron E.J., Tenegold S.M., Bailey and Scott's Diagnostic Microbiology, 8th ed., St Louis: Mosby, (1990)



#### Nitrate Reagent Disks

1. *Acinetobacter calcoaceticus* (left)
2. *Salmonella Typhimurium* (right)

# Nitrocefin Disks

## Cat. No. 49862

Disks to rapidly detect  $\beta$ -lactamase activity in isolated colonies of *Neisseria gonorrhoeae*, *Moraxella catarrhalis*, *Staphylococcus spp.*, *Haemophilus influenzae* and anaerobic bacteria.

### Contents

Each package contains 50 (each 6 mm diameter) filter paper disks impregnated with nitrocefin in a light resistant plastic vial.

### Storage

Store in the freezer below  $-10^{\circ}\text{C}$  in the containers provided. Allow to equilibrate to room temperature before opening and return to freezer storage immediately after use.

### Instructions

Place the required number of nitrocefin disks into a clean, empty Petri dish or onto a microscope slide. Disks may be moistened with one drop of deionized water, but take care not to over-moisten. Using a sterile loop or an applicator stick, remove several similar well-isolated colonies and smear them onto the surface of a disk. Alternatively, moisten the disk with one drop of deionized water and then, holding the disk with forceps, wipe across a colony on an agar plate. Observe the inoculated disk for the development of a red color.

### Interpretation of results

#### Positive

Development of a red color in the area of the disk where the colony was applied. Note the color change does not normally develop over the whole of the disk.

#### Negative

No color change.

A positive result should be interpreted as resistance to penicillin or cephalosporin activity. The susceptibility should be confirmed by standard growth-dependent susceptibility testing methods. A negative result implies but does not guarantee susceptibility.

### Quality control

The table below illustrates the performance of strains used routinely for control.

### User quality control

Check for signs of deterioration. Quality control must be performed with at least one organism to demonstrate a positive reaction and at least one organism to demonstrate a negative reaction. Do not use the product if the reactions with the control organisms are incorrect.

| Test organisms (ATCC®)                | Result   |
|---------------------------------------|----------|
| <i>Haemophilus influenzae</i> (35036) | negative |
| <i>Neisseria gonorrhoeae</i> (31426)  | positive |
| <i>Staphylococcus aureus</i> (11632)  | positive |
| <i>Escherichia coli</i> (25922)       | negative |

### Limitations

It is recommended that biochemical and/or serological tests are performed on colonies from pure cultures to confirm identification.

For most bacterial strains a positive result will develop within 5 minutes. However, a positive reaction for some staphylococci

and anaerobic species may take up to 60 minutes to develop.

Detection of staphylococcal  $\beta$ -lactamase is enhanced by testing growth from a medium containing sub-inhibitory concentrations of a  $\beta$ -lactam antibiotic.

### References

1. MacFaddin J.F., Biochemical Tests for the Identification of Medical Bacteria., 3rd ed. Philadelphia: Lippincott Williams & Wilkins (2000)
2. Murray P.R., Baron E., Pfaller M., Tenover F., Tenover F.C., Manual of Clinical Microbiology., 7th ed. Washington, DC: ASM, (1999)
3. Tu K.K., Jorgensen J.H., Stratton C.W., A Rapid Paper-Disk Test for Penicillinase., Am J. Clin. Pathol., 75:557-9 (1981)
4. Escamilla J., Susceptibility of *Haemophilus influenzae* to Ampicillin as Determined by Use of a Modified One-Minute  $\beta$ -Lactamase Test., Antimicrob. Ag. Chemo., 9:196-8 (1976)
5. Thornsberry C., Biddle J.W., Kirven L.A., Penicillin Resistance in *Neisseria gonorrhoeae* due to  $\beta$ -Lactamase Production., Microbios 20:39-46 (1977)

# ONPG Disks (2-Nitrophenyl $\beta$ -D-galactopyranoside Disks, $\beta$ -Galactosidase Test Disks)

## Cat. No. 49940

Disks to detect the presence of  $\beta$ -galactosidase, an enzyme found in lactose-fermenting organisms

Lactose utilization depends upon two enzymes: a  $\beta$ -galactoside permease, not present in late lactose fermenters, which catalyzes transport of lactose into the cell; and

$\beta$ -galactosidase, which breaks down lactose into galactose and glucose.  $\beta$ -galactosidase is not lactose-specific and can act on simple galactosides including the substrate ONPG (o-nitro- phenyl- $\beta$ -D-galactopyranose). ONPG hydrolysis results in the release of galactose and o-nitrophenol, a yellow chromogen. The test substrate ONPG does not depend on an induced or constitutive permease enzyme to enter the cell. Therefore, the reactions are rapid and occur within a 24-hour period even for late lactose fermenters. The ability to ferment lactose is routinely used to differentiate between *Enterobacteriaceae*.

### Contents

Each package contains 50 sterile filter paper disks of 6 mm diameter, each impregnated with o-nitrophenyl- $\beta$ -D-galactopyranose.

### Instructions

Place one ONPG disk into a sterile test tube. Add 0.1 mL of sterile 0.85% (w/v) sodium chloride solution (physiological saline). Pick up the colony to be tested with a sterile loop and emulsify it in the tube containing the disk and physiological saline. Incubate at 35 °C. To detect active lactose fermenters observe the tube at hourly intervals for up to 6 hours. To detect late lactose fermenters, incubate the negative tubes for up to 24 hours.

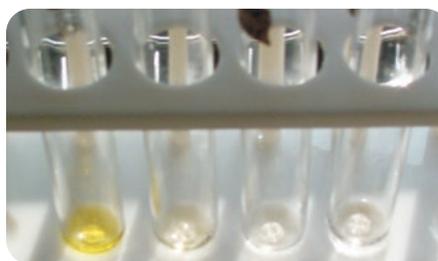
### Quality control

Culture characteristics after growth in a 0.85% (w/v) sodium chloride solution with an ONPG disk for 4 hours at 35 °C.

| Test organisms (ATCC®)                | ONPG hydrolysis |
|---------------------------------------|-----------------|
| <i>Citrobacter freundii</i> (8090)    | +               |
| <i>Enterobacter aerogenes</i> (13048) | +               |
| <i>Escherichia coli</i> (25922)       | +               |
| <i>Proteus vulgaris</i> (8427)        | -               |
| <i>Salmonella arizonae</i> (13314)    | +               |
| <i>Salmonella Typhimurium</i> (14028) | -               |

### References

1. W.L. Gaby, C. Hadley, J. Bact., 74, 356 (1957)  
J. Sanbrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual 2nd ed., Cold Spring Harbor, NY (1989)
2. S.R. Maloy, J.E. Conran (Jr.), D. Freifelder, Microbial Genetics 2nd ed. Jones and Bartlett Boston, MA (1994)
3. V.E. Becker, H.J. Evans, The influence of Monovalent Cations and Hydrostatic Pressure on  $\beta$ -Galactosidase Activity., Biochim. Biophys. Acta, 191, 95 (1969)
4. M.C. Neville, G.N. Ling, Synergistic Activation of  $\beta$ -Galactosidase by Na<sup>+</sup> and Cs<sup>+</sup>., Arch. Biochem. Biophys., 118, 596, (1967)
5. J. Lederberg, The  $\beta$ -Galactosidase of *Escherichia coli*, strain K-12., J. Bact., 60, 381 (1950)



**ONPG Disks (yellow = positive reaction, clear = negative reaction)**

# Optochin Disks

## Cat. No. 74042

### A test for the identification of or differentiation between pneumococci and viridans streptococci

Optochin (ethyl hydrocuprein hydrochloride) is inhibitory for pneumococcal growth whereas other streptococci show good growth or a very small zone of inhibition. Further tests are required for the diagnosis of pneumococcal infections, since  $\alpha$ -hemolytic (viridans) streptococci and pneumococci (*Streptococcus pneumoniae*) cannot be easily differentiated on a blood agar plate (showing both a partial clearing of blood and a greenish discoloration;  $\alpha$ -hemolysis). The optochin test disks are suitable for the examination of specimens like sputum, pleural fluid, lung aspirate, urine or blood. The correlation between bile solubility and full optochin susceptibility for the differentiation of *Streptococcus pneumoniae* from other streptococci was shown by Bowers and Jeffries<sup>1</sup>.

### Contents

Each package contains 50 sterile filter paper disks impregnated with optochin.

### Instructions

Gram staining test (Cat. No. 77730) is required, followed by the optochin sensitivity test. Prepare tryptone soya agar (Cat. No. 22091) with blood or blood agar (Cat. No. 70133) plates and streak pure culture of the organism to be tested across one half of the plate. Streak a known *Pneumococcus* culture across the other half of the plate as positive control and immediately place the optochin disks in the center of the two halves of the plate and incubate at 37 °C. Observe for zone of inhibition around the disks.

### Quality control

Culture response after 18–24 hours at 35–37 °C on seeded tryptone soya agar (Cat. No. 22091) with blood using optochin disks.

| Test organisms (ATCC®)                 | Diameter of zone of inhibition                                    |
|--|---|
| <i>Streptococcus pneumoniae</i> (6303) | 15 mm   |
| <i>Streptococcus pyogenes</i> (19615)  | resistant to optochin, no or marginal zone inhibition (</= 13 mm) |

### References

1. E.F. Bowers, L.R. Jeffries, J. Clin. Path., 8, 58 (1995)
2. Bouvet A., Grimont F., Grimont P.A.D., *Streptococcus defectivus* sp. nov. and *Streptococcus adjacens* sp. nov., Nutritionally Variant Streptococci from Human Clinical Specimens., Int. J. Syst. Bacteriol., 39, 290 (1989)
3. Baron E.J., Peterson L.R., Finegold S.M., Bailey and Scott's Diagnostic Microbiology., 9th edition. St. Louis, Mosby (1994)

# Oxidase Strips

## Cat. No. 40560

### A test to detect cytochrome oxidase activity in microorganisms within a minute

The oxidase strip test is an important differential procedure which can be performed on all Gram-negative bacteria that are to be identified. The cytochrome oxidase enzyme present in most Gram-negative bacteria triggers the reaction of N,N-dimethyl-p-phenylenediamine with  $\alpha$ -naphthol, forming indophenol blue.

#### Contents

The kit contains 100 plastic strips with a paper zone saturated with a solution of N,N-dimethyl-1,4-phenylenediamine and  $\alpha$ -naphthol.

#### Storage

Store dry at +2 to +8°C. The shelf life of this product can be extended by storing it at -20°C.

#### Instructions

Wipe off several suspect colonies from a Petri dish with the active zone of a diagnostic strip. Read result after one minute.

For accurate results, store dry. The aluminum tube containing the strips must not be opened before the temperature has equilibrated to room temperature to prevent condensation of air humidity onto the strips. However, test performance requires that there is sufficient humidity on the cultures or the cultivation media where the suspect colonies are tested. If sufficient moisture is not available, the active zone of strip can be moisturized by using water that has condensed on to the lid of the dish or by adding approximately 10  $\mu$ L of distilled water. Do not wipe the colonies with a microbiological loop made of metal.

#### Principle and interpretation of results

Gordon and McLeod<sup>1</sup> introduced the oxidase tesgonococci based upon the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and  $\alpha$ -naphthol. Later, Gaby and Hadley<sup>2</sup> introduced a more sensitive method by using N,N-dimethyl-p-phenylenediamine oxalate, establishing that staphylococci are Gram-negative. The cytochrome oxidase present in most Gram-negative bacteria triggers the reaction of N,N-dimethyl-p-phenylenediamine with  $\alpha$ -naphthol, forming indophenol blue.

#### The oxidase test is mainly used to differentiate

1. Oxidase positive *Neisseria* from other Gram-negative diplococci
2. Oxidase positive *Aeromonas hydrophila* from *Escherichia coli* (Gram-negative)
3. Oxidase positive *Plesiomonas shigelloides* from *Shigella sonnei* (Gram-negative)

Cytochrome oxidase production may be inhibited by acid production, and a false negative reaction may be given by *Vibrio*, *Aeromonas*, and *Plesiomonas* spp. when grown on a medium containing fermentable carbohydrate such as MacConkey Agar (Cat. No. 70143). Colonies taken from media containing nitrate may give unreliable results. The loss of activity of the oxidase reagent is caused by autoxidation which may be avoided by adding 0.1% ascorbic acid (Cat. No. 95209).

#### Negative reaction

No color change develops at the position of the colony on the strip.

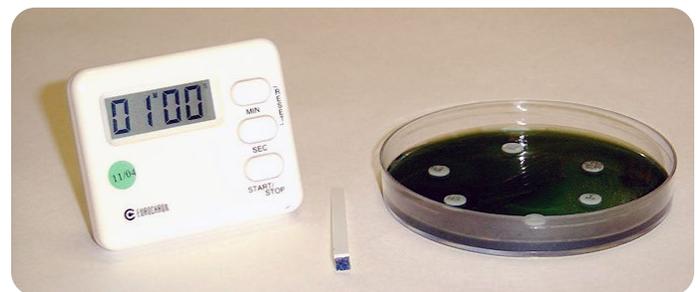
#### Positive reaction

A dark blue or black spot develops at the position of the colony on the strip.

#### Quality control

The list below illustrates control strains in routine use.

| Test organisms (ATCC®)                | Result   |
|---------------------------------------|----------|
| <i>Escherichia coli</i> (25922)       | negative |
| <i>Pseudomonas aeruginosa</i> (27853) | positive |



Oxidase Strips

#### References

1. J.Gordon, J.W. McLeod, J. Path.Bact., 31, 185 (1928)
2. W.L. Gaby, C. Hadley, J. Bact., 74, 365 (1957)
3. K.J. Steel, J. Appl. Bact., 25, 445 (1962)

# Oxidase Test Disks

## Cat. No. 70439

An important differential test that can be performed on all Gram-negative bacteria to be identified

### Contents

Each package contains 50 disks impregnated with N,N-dimethyl-p-phenylenediamine oxalate and  $\alpha$ -naphthol.

### Storage

Store dry at +2 to +8 °C. The shelf life of this product can be extended by storing it at -20 °C.

### Instructions

With an inoculating loop or a toothpick, touch and spread a well isolated colony on an oxidase disk. The reaction is observed within 2 minutes at 25–30 °C.

Principle and interpretation of results Gordon and McLeod<sup>1</sup> introduced the oxidase test for identifying gonococci based upon the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and  $\alpha$ -naphthol. Later, Gaby and Hadley<sup>2</sup> introduced a more sensitive method by using N,N-dimethyl-p-phenylenediamine oxalate, establishing that staphylococci are Gram-negative. The cytochrome oxidase present in most Gram-negative bacteria triggers the reaction of N,N-dimethyl-p-phenylenediamine with  $\alpha$ -naphthol, forming indophenol blue.

### The oxidase test is mainly used to differentiate

1. Oxidase positive *Neisseria* from other Gram-negative diplococci.
2. Oxidase positive *Aeromonas hydrophila* from *Escherichia coli* (Gram-negative)
3. Oxidase positive *Plesiomonas shigelloids* from *Shigella sonnei* (Gram-negative)

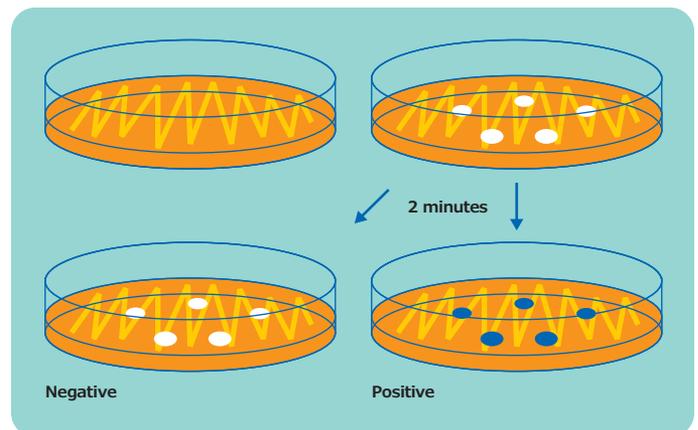
Cytochrome oxidase production may be inhibited by acid production, and a false negative reaction may be given by *Vibrio*, *Aeromonas*, and *Plesiomonas* species when grown on a medium containing fermentable carbohydrates such as MacConkey Agar (Cat. No. 70143). Colonies taken from media containing nitrate may give unreliable results. The loss of activity of the oxidase reagent is caused by autoxidation which may be avoided by adding 0.1% ascorbic acid (Cat. No. 95209).

Reaction takes place within 2 minutes at 25–35 °C.

| Test organisms (ATCC®)                | Growth   | Nitrate reduction |
|---------------------------------------|----------|-------------------|
| <i>Pseudomonas aeruginosa</i> (27853) | positive | deep purple blue  |
| <i>Staphylococcus aureus</i> (25923)  | negative | –                 |
| <i>Neisseria gonorrhoeae</i> (19424)  | positive | deep purple blue  |
| <i>Escherichia coli</i> (25922)       | negative | –                 |

### References

1. J.Gordon, J.W. McLeod, J. Path.Bact., 31, 185 (1928)
2. W.L. Gaby, C. Hadley, J. Bact., 74, 365 (1957)
3. K.J. Steel, J. Appl. Bact., 25, 445 (1962)



Oxidase Disks

# PYrase Strips (Pyrrolidonyl Peptidase Strips)

## Cat. No. 67886

Test for the rapid differentiation of enterococci from group D streptococci and differentiation of *Streptococcus pyogenes* from other hemolytic streptococci.

### Contents

The kit contains 50 plastic strips with an active zone saturated with chromogenic substrate for the detection of pyrrolidonyl peptidase, and 1.0 mL of developing reagent.

### Storage

Store dry at +2 to +8 °C. The shelf life of this product can be extended by storing it at -20 °C.

### Instructions

Wipe off several suspect colonies from a Petri dish with the active zone of diagnostic strip. After one minute, apply approximately 10 µL of developing reagent to the active zone, using a plastic loop or micropipette. The reaction is observed within 1 minute.

### Interpretation of results

#### Negative reaction

No color change develops at the position of the colony on the strip.

#### Positive reaction

A red spot develops at the position of the colony on the strip.

The PYrase test is recommendation by NRL for streptococci and enterococci .



PYrase Strips

### References

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2. R.R. Facklam, L.G. Thacker, B. Fox, L. Eriquez, Presumptive Identification of Streptococci with a New Test System, J. Clin. Micro., 15, 987 (1982)
3. B.L. Wasilaukas, K.D. Hampton, Evaluation of the Strep-A-Fluor Identification Method for Strep A Streptococci, J. Clin. Micro. 20, 1205 (1984)
4. D.M. Yajko, J. Lawrence, P. Nassos, J. Young , K.W. Hadley, Clinical trial comparing bacitracin with Strp-A-Chek for accuracy and turnaround time in the presumptive identification of Streptococcus pyogenes, J. Clin. Micro., 24, 431 (1986)
5. L.P. Gordon, M.A.S. Damm, J.D. Anderson, Rapid Presumptive Identification of Streptococci Directly from Blood Cultures by Serologic Tests and the L-Pyrrolidonyl-β-Naphthylamide Reaction, J. Clin. Micro. 25, 238 (1987)
6. P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, R.H. Tenover, Eds. Manual of Clinical Microbiology, 7th ed., Washington, D.C., ASM Press (1999)
7. J.F. MacFaddin, Biochemical tests for the Identification of Medical Bacteria, 3rd ed., Philadelphia, Lippincott Williams & Wilkins (2000)

| Positive (change to red color) | Negative (no color change)                       |
|--------------------------------|--|
| <i>Enterococcus</i>            | <i>Streptococcus bovis</i>                       |
| <i>Streptococcus pyogenes</i>  | <i>Streptococcus equinus</i>                     |
| <i>Lactococcus lactis</i>      | Minute beta hemolytic streptococci:              |
| <i>Aerococcus</i>              | <i>Streptococcus anginosus</i>                   |
| <i>Gamella</i> (most strains)  | <i>Streptococcus intermedius</i>                 |
|                                | <i>Streptococcus constellatus</i>                |
|                                | Other <i>Lactococcus</i> species                 |
|                                | <i>Leuconostoc</i>                               |
|                                | <i>Pediococcus</i>                               |
|                                | <i>Lactobacillus</i><br>(sporadic positive test) |
|                                | Other species of viridans streptococci           |

# Sterility Indicator (Steam Sterilization)

## Cat. No. 74041

Indicator strips that harbor  $10^6$  *Bacillus stearothermophilus* (ATCC® 7953) spores

*Bacillus stearothermophilus* is a thermophilic species that can grow at temperatures of 65 °C and higher. Its spores are an excellent tool to monitor autoclave performance, as they are highly resistant to temperature. These indicators are similar to those used to fulfill cGMP requirements by the US FDA.

### Contents

A package contains 25 test strips individually bagged in an envelope. Each paper strip is impregnated with  $10^6$  spores of *Bacillus stearothermophilus* (ATCC® 7953).

### Instructions

Place the indicators in such a manner that they are exposed to the steam in representative places among the media and the equipment. For routine evaluation of each autoclaved lot, a standard procedure should be established. After steam sterilization open the envelope containing the strip using strictly aseptic techniques. Then inoculate the strip in a tube with sterile CASO broth (Cat. No. 22098) and incubate at 55–60 °C for up to 7 days. For control purposes, an unexposed spore strip should be inoculated at the same time in another tube with sterile CASO broth.

### Quality control

Culture response observed after 7 days at 55–60 °C in CASO Broth (Cat. No. 22098):

| Spore strip         | Result    |
|---------------------|-----------|
| Exposed to steam    | no growth |
| Unexposed Luxuriant | growth    |

# Sterile Disks

## Cat. No 74146

Disks to test a variety of antibiotics, carbohydrates, substrates, and antiseptics on bacteria in Petri dishes

The sterile disks can be used to test a variety of antibiotics, carbohydrates, substrates, and antiseptics on bacteria in Petri dishes. Soak a disk in the solution or apply some solution on the disks. Allow to dry, then place the disk into an inoculated agar plate. Each disk will absorb exactly the same amount of liquid.



Sterile Disks

# Sterility Indicator (Radiation Sterilization)

## Cat. No. 05290

Indicator strips that harbor  $10^6$  *Bacillus pumilus* (ATCC® 27142) spores

These indicators have *Bacillus pumilus* (ATCC® 27142) spores impregnated on individually bagged paper strips. The number of spores impregnated onto each strip is approximately one million. *Bacillus pumilus* is a radiation resistant species. The highly resistant spores are used to monitor the efficiency of sterilization by radiation.

### Contents

A package contains 25 test strips individually bagged in an envelope. Each paper strip is impregnated with about  $10^6$  spores of *Bacillus pumilus* (ATCC® 27142).

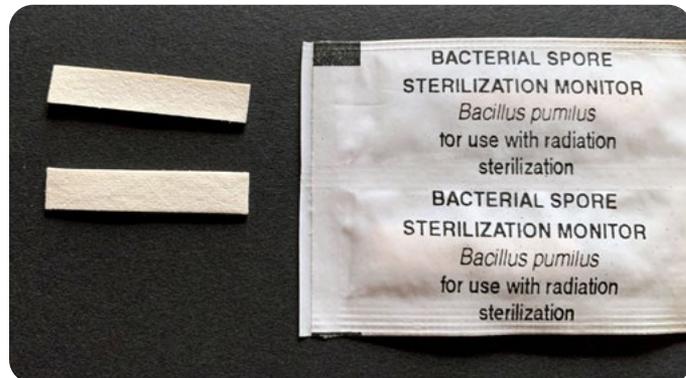
### Instructions

Place indicators in such a manner that they are exposed to the same radiation as the media and the equipment. For the routine evaluation of each radiated lot, a standard procedure should be established. After sterilization by radiation, open the envelope containing the strip using strictly aseptic techniques. Then inoculate strip in a tube with sterile CASO broth (Cat. No. 22098) and incubate at 35–37 °C for up to 7 days. For control purposes, an unexposed spore strip should be inoculated at the same time in another tube with sterile CASO broth.

### Quality control

Culture response observed after 7 days at 35 °C in CASO Broth (Cat. No. 22098):

| Spore strip          | Result           |
|----------------------|------------------|
| Exposed to radiation | no growth        |
| Unexposed            | Luxuriant growth |



Sterility Indicator

# Tributyryn-Strips (TRIBU Strips)

## Cat. No. 75744

### A test for the differentiation of *Branhamella* and *Neisseria*

The test is based on the enzymatic hydrolysis of tributyrin, a reaction that causes the color change of an acid-base indicator. The results can be read after 18–20 hours.

#### Composition

Each package contains 300 test strips saturated with tributyrin, and an acid-base indicator.

#### Storage

Store dry at +2 to +8 °C. The shelf life of this product can be extended by storing it at –20 °C.

The strips are delivered sterile. Make sure to maintain sterility when the container is repeatedly opened. To obtain accurate results, avoid moisture during storage. Allow to equilibrate to room temperature before opening the container. If this is not observed, the product can become moistened by condensation and will deteriorate.

#### Instructions

Using a sterile forceps, throw one tributyrin strip into a 1 mL suspension of the test strain in buffered saline (pH 7.2). Incubate the test sample at 37 °C (without CO<sub>2</sub>). Preliminary results can be read after several hours if the red color changes to yellow (positive result). The final result can be read after 18–20 hours of incubation.

#### Interpretation of results

##### Negative reaction

Red color has not changed to yellow (*Neisseria*)

##### Positive reaction

Red color has changed to yellow (*Branhamella*)

#### Identification diagram for *Branhamella*

| Tributyryn reduction of nitrates |                             |
|----------------------------------|-----------------------------|
| +                                | -                           |
| <i>Branhamella</i>               | <i>Neisseria</i>            |
| saccharides +/-                  | saccharides +/-             |
| β-lactamase +/-                  |                             |
| +<br>adapted parasite            | -<br>opportunistic pathogen |

#### Quality control

The list below illustrates control strains in routine use.

| Test Organisms (ATCC®)                 | Result   |
|--|----------|
| <i>Neisseria gonorrhoeae</i> (19424)   | negative |
| <i>Branhamella catarrhalis</i> (25238) | positive |

#### References

- Berger U., Über die Spaltung von tributyrin durch *Neisseria*., Arch. Hyg. Bakteriol., 146:388-391 (1962)
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- August M.J., et al., Cumitech 3A; Quality Control and Quality Assurance Practices in Clinical Microbiology, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington D.C. (1990).
- Perez J.L., et al. Butyrate Esterase (Tributyryn) Spot Test, a Simple Method for Immediate Identification of *Moraxella (Branhamella) catarrhalis*. J. Clin. Microbiol., 28: 2347-2348 (1990)
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- Koneman E.W., et al. Color Atlas and Textbook of Diagnostic Microbiology, 5th ed. J.B. Lippincott Company, Philadelphia, PA, (1997)
- Forbes B.A., et al. Bailey and Scott's Diagnostic Microbiology, 10th ed. C.V. Mosby Company, St. Louis, MO, (1998)



Tributyryn-Strips reactions

# Haemophilus Differentiation Disks

## X Factor / V Factor / X + V Factors

Cat. No. 77148 / 89788 / 08482

Presumptive identification of *Haemophilus* species based on requirement for X or V growth factor or both

The members of the genus *Haemophilus* require hemin (X factor) and/or nicotinamid- adenin-dinucleotid (V factor) to grow. The need for either one or both factors provides a way to differentiate between these organisms.

### Content

Each package contains 50 test disks. The sterile 6 mm diameter filter paper disks are impregnated with either:

- Hemin and nicotinamide adenine dinucleotide (X and V factor disks; Cat. No. 08482).
- Hemin (X factor disks; Cat. No. 77148).
- Nicotinamide adenine dinucleotide (V factor disks; Cat. No. 89788).

### Instructions

Inoculate the surface of a blood agar (Cat. No. 70133) or brain heart infusion agar plate (Cat. No. 70138) with the test organisms, either by streaking or surface spreading. Aseptically place the X and V factor disks on the plate. Incubate the plates at 35–37 °C for 24–48 hours.

### Recommended disk positions on the agar plate

| Disk              | Position   |
|-------------------|------------|
| X factor disk     | 12 o'clock |
| V factor disk     | 4 o'clock  |
| X + V factor disk | 8 o'clock  |

Observe the growth around the disks. Test organisms requiring X factor grow only in the vicinity of X disks. Those that require V factor grow only in the vicinity of V disks. The ones that require both the V and X factors grow only in the vicinity of disks containing both X and V factor.

Note: Use known strains of *Haemophilus influenza* to monitor the performance of the disks and the medium. Do not use a heavy suspension of the test organisms as carry over of X or V factor from the primary growth medium may take place.

### Quality control

Culture response observed on brain blood agar (Cat. No. 70133) plate or brain heart infusion agar (Cat. No. 70138) after 24–48 hours at 35–37 °C.

| Test organisms (ATCC®)                  | Without growth factor | X factor | V factor | X +V factor |
|---|-----------------------|----------|----------|-------------|
| <i>Haemophilus influenzae</i> (35056)   | -                     | -        | -        | +           |
| <i>Haemophilus parainfluenza</i> (7901) | -                     | -        | +        | +           |
| <i>Haemophilus ducreyi</i> (27722)      | -                     | +        | -        | +           |
| <i>Bordetella pertussis</i> (13048)     | +                     | +        | +        | +           |

### References

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## Looking for a rapid method for microbial identification?

Smart, inexpensive membranes can help to identify organisms within 1 to 4 hours.

These substrate-carrying ID membranes, which are simply placed onto the agar surface after routine incubation, allow the identification and differentiation of colonies by means of characteristic color reactions. This economical and rapid identification and confirmation method is suitable for microorganisms from water, food, environmental and clinical samples. It is used in various sectors, including the food, dairy, water and cosmetics industries, pharmaceutical laboratory testing, environmental and sanitary testing, and clinical diagnostics.

### Principle

The membranes contain chromogenic substrates such as ONPG, X-Gal, or X-Glu, and other substrates and indicators which serve as the basis for microbe differentiation by color. The target organisms possess enzyme systems that metabolize the substrates in the membrane, leading to a change of color. The colors can be visually detected on the membrane. This indicates microbial enzymatic activity which serves to identify the genus and species. No reagents or liquids need to be added. If the membrane is removed from the plate after replication of the colony pattern, the plate can be used for further tests or to streak out colonies onto different plates.

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