Analytix *Notes*

Disks and Strips for Microbiology

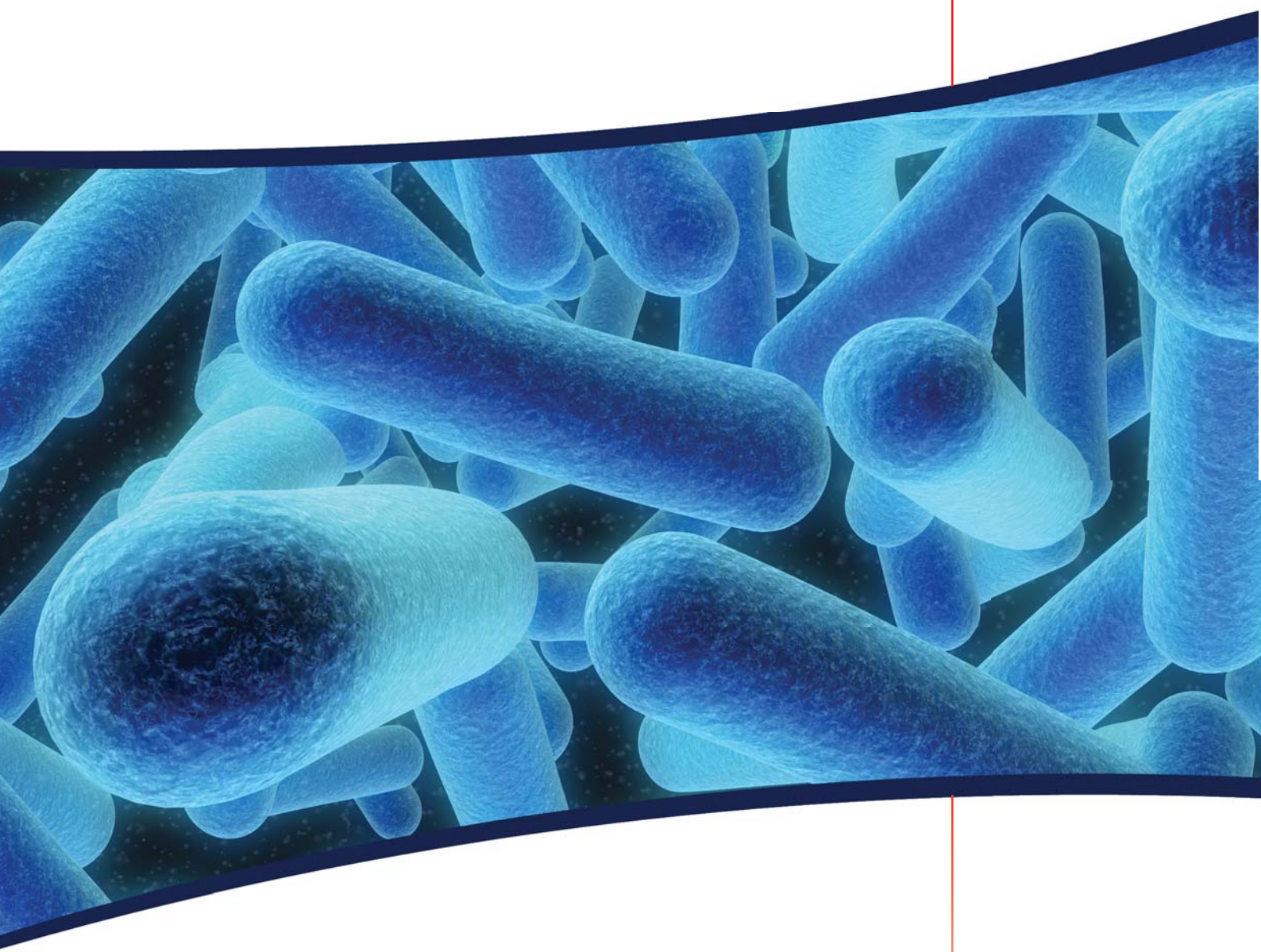

Table of contents

Disks and Strips for Microbiology ..... Disks and Strips are very helpful for identification and confirmation of microorganisms or to monitor sterilization. They are based on rapid methods, are easy to prepare and have a very good price/quality relationship.

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 using chromogenic substrates or on complex building reactions. Also, sensitivity to certain inhibitory substances can be also be tested for.

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Fluka Cat. No. 75554 Aminopeptidase Test (Gram-Positive Test) .....

The aminopeptidase test is intented for use in the detection of Gram-positive microorganisms by checking 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.

Instructions

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

Note: The growth medium from which the colonies were taken should not contain any dyes or indicators. The use of 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 behaviour, however, it does not replace Gram-staining, as it cannot show morphology.

Organisms	L-alanine aminopeptidase
Gram-negative bacteria (Exceptions: *Bacteroides vulgatus, Bacteroides fragilis, Campylobacter sp., Veillonella parvula)*	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) 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)

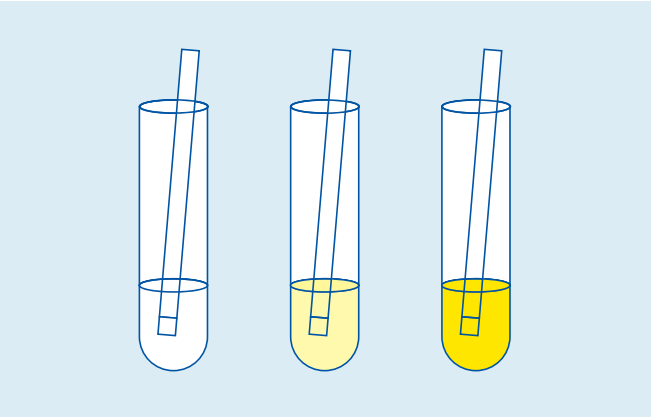
(3) 3. J.D. Costin, M. Kappner, W. Schmidt: Differenzierung von Gram-positiven Bakterien und Gram-negativen Bakterien mit dem L-Alanin Aminopeptidase Test, Forum Mikrobiolo., 351 (1983)

(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-Positve Bacteria, Eur. J. Appl. Microbiol. Biotechnol., 5, 113 (1978)

(6) I. Otte, A. Tolle, Aminopetidase- und Gram-Reaktion von Bakterien, Milchwiss., 35, 215 (1980)

Picture ..... Aminopeptidase Test
(from left to right)
1. Negative Reaction
2. Postive Reaction
3. Postive Reaction



Fluka Cat. No. 08382 Bacitracin Disks ..... The bacitracin disks are used in the presumptive identification of group A β-hemolytic Streptococci and allow the differentiation of group A β-hemolytic Streptococci from other β-hemolytic Streptococci.

The bacitracin disks are used in the presump-
Streptococci ation of group A β-hemolytic *Streptococci* and allow tStreptococci ation of group A β-hemcStreptococci:occi from other β-hemolytic *Streptococci*. The bacitracin test should be performed together with the SXT susceptibility test (Fluka Cat. No. 73477), as their combined results increase the sensitivity and the predictive value of the bacitracin test.

Composition

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

Storage

Store in the freezer below –18°C in the containers provided. Allow to equilibrate to room temperature before opening. Return to freezer storage immediately after use.

Instructions

Prepare blood agar (Fluka Cat. No. 70133) plates and inoculate the plates with the suspect organism using surface spreading technique to obtain confluent growth. Place the bacitracin disks aseptically onto the inoculated surface and press gently. Invert the plates and incubate at 35-37°C in 5-10 % CO_2 for 18-24 hours, 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 iiStreptococci ceptibility 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 35-37°C for 18-24 hours).

Test Organisms (ATCC)	Diameter of zone of inhibition
Streptococcus pyrogenes (19615)	15 mm
Streptococcus agalactiae (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)

07773 Bacteriuria Test Kit (Nitrate Reagent Test Kit; Urine Nitrite Test Kit; Nitrite Indicator Strips Kit)..... rapid detection of nitrate reduction in urine.

This test can be used to detect the presence of nitrite, which indicates the presence of bacteria that may be caused by infection of the kidneys, urethra, or bladder. Urine from healthy humans contains no nitrite.

Composition:

One box contains 5 0 indicator strips (Fluka 05857) and one vial containing 5 ml of potassium nitrate solution (Fluka 03717).

Strips contain:

a-Naphtylamine
Citric Acid
Sulfanilic Acid

Attention:

Store indicator strips in a cool (2–8 °C), light-protected place.

Directions:

1. Fresh urine (no older than four hours) is required. The first morning urine is preferable.
2. Place one drop of urine on strip. Color change to purple indicates presence of bacterinuria. **If color change is not observed, additional assay must be conducted:**
3. Add 50 µL of potassium nitrate solution (provided with kit) to vial containing one mL of fresh urine.
4. Incubate vial at 37 °C for one hour.
5. Place one drop of incubated urine on incubator strip.
6. No color change indicates negative bacterinuria result.

Principle and Interpretation:

In the presence of nitrate-reducing bacteria in human urine, nitrite is formed and reacts with sulfanilic acid to aid formation of diazotized sulfanilic acid. The product of this formation reacts with a-naphtylamine to yield an azoderivate that is purple in color. The incubation of urine with potassium nitrate increases the sensitivity of the assay (to detect lower level of bacteria). Additional growth of bacteria produces additional amounts of nitrite. This test kit allows for the detection of bacteria even in cases of lower bacteria concentrations.

Limitation of Test:

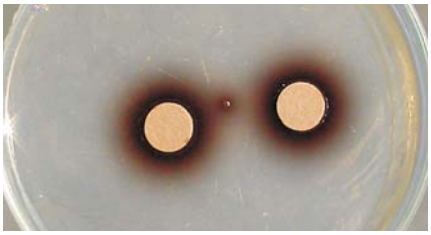
Bacteria detected by this approach: *E. coli, Proteus sp., Klebsiella, Aerobacter, Citrobacter, Salmonella*
Not detected: *Pseudomonas*, Streptococci, Staphylococci

Test Organisms (ATCC)	Nitrate reduction
Salmonella serotype typhimurium (14028)	+
Escherichia coli (25922)	+
Pseudomonas aeruginosa (27853)	-

References:

(1) European Pharmacopeia II, Kapitel VIII, 10.
(2) Pelczar, M. J., Jr.; Reid, R. D. Microbiology, 2nd ed.; McGraw-Hill: New York, 1965; p 567.
(3) Stainer, R. Y.; Douderoff, M.; Adelberg, E. A. *The Microbial World*, 2nd ed.; Prentice-Hall: New York, 1963; pp 116–117.
(4) Wideman, P. A.; Citronbaum, D. M.; Sutter, V. L. Simple Disk Technique for Detection of Nitrate Reduction in Anaerobic Bacteria. *J. Clin. Micro.* 1977, 5 (3), 315–19.
(5) Lennette, E. H.; Balows, A.; Hausler, W. J.; et al. *Manual of Clinical Microbiology*, 4th ed.; Washington, DC: ASM, 1985.
(6) Baron, E.J.; Finegold, S.M. *Bailey and Scott's Diagnostic Microbiology*, 8th ed.; Mosby: St Louis, 1990.

Fluka Cat. No. 80507 Bile Esculin Disks (Esculin Bile Disks Kit;) ..... The bile esculin disks are used for rapid detection of esculin hydrolysis in presence of bile.



Picture ..... Bile Esculin Disks

The detection of esculin hydrolysis in presence of biles allows the differentiation of group D Streptococci 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, being a blackish-brown coloured complex formed.

Composition

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

Instructions

Place a bile esculin disk on the seeded bile esculin agar base (without esculin) plate or another media. Incubate at 35°C for 18-24 hours.

Quality control

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

References

(1) Rochaix, C.R.Soc. Biol., 90, 771 (1924)

(2) Meyer and Schönfeld, Zentralbl. Bacteriol. Parasitenkd. Infectionskr. Hyg. Abt. I Orig., 99, 402 (1924)

(3) 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)

Test Organisms (ATCC)	Esculin Hydrolysis
Streptococcus faecalis (29212)	+
Streptococcus pyrogenes (19615)	–
Listeria monocytogenes (19118)	+

Carbohydrates Differentiation Discs..... Carbohydrates Differentiation Discs are used to differentiate bacteria on the basis of carbohydrate fermentation abilities.

The following carbohydrates are available as differentiation discs:

Composition:

One vial contains 25 sterile filter-paper discs impregnated with carbohydrates.

Any liquid, semisolid, or solid media can be used. Liquid and semisolid media are dispensed in 5 ml aliquots in test tubes for observation of fermentation. A single car-bohydrate disc inoculated with test organisms is added to each tube. With semisolid

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

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

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

Liquid Media

Andrade Peptone Water (Sigma/Fluka A0715, Fluka 28943)
Andrade Peptone Water with Meat Extract
Phenol Red Broth Base (Sigma/Fluka P8976)
Phenol Red Broth with Meat Extract
Purple Broth
Yeast Fermentation Broth

Semisolid Media

Cystine Tryptone Agar
OF Basal Medium (Fluka 75315)
Tryptone Agar

Solid Media

Phenol Red Agar
Purple Agar Base
Sanders Agar

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 same plate. Sterile plates containing the agar medium of choice are surface-seeded with test organism(s), and required car-bohydrate discs are pressed gently into the medium surface at a minimum distance of 2 cm apart. Incubation is carried out at 36 °C (± 1.0 °C) for 18–48 hours. Results are recorded once at 18–24 hours and again at 48 hours. Test plates should be observed 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, gas produced is trapped and seen as bubbles. On agar plates, fermentation appears as a change in color around the disc.

Microorganisms can only ferment certain carbohydrates. Depending on the enzymes they possess, they are able to cleave different carbohydrates. This process can be detected by gas production (CO_2) in liquid media and/or color change in 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
Citrobacter freundii (8090)	luxuriant	-	-	+	+	+	-	+	+	-	-			+	+
Enterobacter aerogenes (13048)	luxuriant	+	+	+	+	+	+	+	+	-	-	+	+	+	+
Escherichia coli (25922)	luxuriant	-	-	+	+	-	-	+	+	-	-	+	+	+	+
Klebsiella pneumoniae (13883)	luxuriant	+	+	+	+	+	+	+	+	-	-	+	+	+	+
N. meningitis (13090)	luxuriant							+	-			-	-	-	-
Proteus vulgaris (13315)	luxuriant	-	-	-	-	-	-	+	+	-	-	-	-	+	+
Salmonella typhimurium (14028)	luxuriant	-	-	+	+	-	-	+	+	+	+	+	+	+	+
Salmonella typhi (6539)	luxuriant	-	-	-	-	-	-	+	-	-	-	-	-	+	-
Serratia marcescens (8100)	luxuriant	-	-	-	-	-	-	+	+	-	-	+	-	+	-
Shigella flexneri (12022)	luxuriant	-	-	-	-	-	-	+	-	-	-	+	+	+	-
Strep. pneumoniae (6303)	luxuriant	-	-	+	-	-	-	+	-	-	-	+	+	+	-
Strep. pyogenes (19615)	luxuriant							+	-						

Organisms (ATCC)	Growth	Inositol		Inulin		Lactose		Maltose		Mannitol		Mannose		Melibiose	
		Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Citrobacter freundii (8090)	luxuriant	-	-			+	+	+	+	+	+	+	+	-	-
Enterobacter aerogenes (13048)	luxuriant	+	+			+	+	+	+	+	+	+	+	+	+
Escherichia coli (25922)	luxuriant	-	-			+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae (13883)	luxuriant	+	+			+	+	+	+	+	+	+	+	+	+
N. meningitis (13090)	luxuriant							+	-						
Proteus vulgaris (13315)	luxuriant	-	-			-	-	+	+	-	-	-	-	-	-
Salmonella typhimurium (14028)	luxuriant	+	+			-	-	+	+	+	+	-	-	+	+
Salmonella typhi (6539)	luxuriant	-	-			-	-	+	-	+	-	+	+	+	+
Serratia marcescens (8100)	luxuriant	+	-			-	-	+	-	+	-	+	+	-	-
Shigella flexneri (12022)	luxuriant	-	-			-	-	+	-	+	-	+	+	-	-
Strep. pneumoniae (6303)	luxuriant			+	-	+	-	+	-						
Strep. pyogenes (19615)	luxuriant			-	-			+	-						

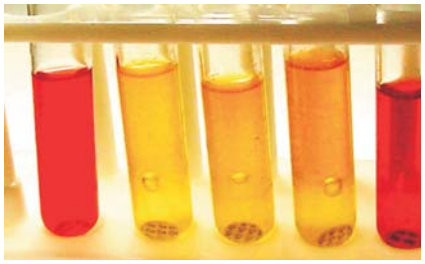
Organisms (ATCC)	Growth	Raffinose		Rhamnose		Salicin		Sorbitol		Sucrose		Trehalose		Xylose	
		Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Citrobacter freundii (8090)	luxuriant	-	-	+	+	-	-	+	+	+	+	+	+	+	+
Enterobacter aerogenes (13048)	luxuriant	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Escherichia coli (25922)	luxuriant	-	-	+	+	-	-	+	+	-	-	+	+	+	+
Klebsiella pneumoniae (13883)	luxuriant	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N. meningitis (13090)	luxuriant				+					-	-				
Proteus vulgaris (13315)	luxuriant	-	-	-	-	+	+	-	-	+	+	+	+	+	[+]
Salmonella typhimurium (14028)	luxuriant	-	-	+	+	-	-	+	+	-	-	+	+	+	+
Salmonella typhi (6539)	luxuriant	-	-	-	-	-	-	+	-	-	-	+	-	+	-
Serratia marcescens (8100)	luxuriant	-	-	-	-	+	[+]	+		+	+	+	[+]		
Shigella flexneri (12022)	luxuriant	-	-	-	-	-	-	+	-	-	-	+	-	-	-
Strep. pneumoniae (6303)	luxuriant									+	-				
Strep. pyogenes (19615)	luxuriant					+	-			+	-				

Key:

(§) longer if necessary
\+ positive reaction, color changes to yellow
\- negative reaction, no color change or remains red
[+] weak/slight reaction
* 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:

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



Picture ..... Carbohydrate Disks

Fluka Cat. No. 75832 Coagulase Test (Slide) ..... The enzyme coagulase, produced by a few of the *Staphylococcus* species, is a key feature of pathogenic staphylococci.

For the detection of coagulase-negative or -positive organisms (Staphylococcus aureus).

Product Description:
1 box contains 30 discs (made from rabbit plasma)

Store below 8°C and use before expiry date.

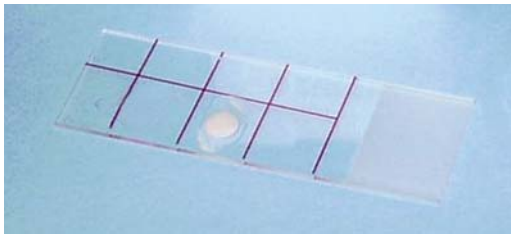
Directions:
Place 1 drop of distilled water on a glass microscope slide and prepare a heavy suspension of the organism being tested. Add 1 Coagulase Disc and rub the disc about in the suspension, using the tip of a wire loop. At once add a second drop of distilled water and mix again.

Coagulase-negative organisms will remain evenly suspended. Macroscopic clumping will occur within 30 seconds in a positive test; do not observe beyond 1 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 the detection of coagulase. It is thought that an infective organism that produces the coagulase enzyme may protect itself by inducing clotting in surrounding tissues, thereby inhibiting destruction by normal body defenses such as phagocytosis or antibodies. The coagulase formation by *Staphylococcus aureus* and its formation of enteric toxins 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* produce 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 a- and b-chains of the plasma fibrinogens to form a coagulate. With this test only the bound form of coagulase is measured.

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)



Picture ..... Coagulase Test Disc Strip

Fluka Cat. No. 05686 DMACA Indole Disks (Indol Detection Disks) .....

The DMACA indole disks are used to determine the ability of an organism to split tryptophan into indole and α-aminopropionic acid.

The DMACA indole disks are used to determine the ability of an organism to split tryptophan into indole and α-aminopropionic acid. The presence of indole can be detected by adding DMACA, that gives rise to a blueish-purple complex. Using this method it is possible to differentiate *Escherichia coli* from *Klebsiella*.

Contents

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

Instructions

Place the disk on a suspect colony grown in a media such as modified HiCrome UTI agar (Fluka Cat. No. 16636) or Christensen's urea agar (Fluka Cat. No. 27048). Lay the Petri dish against a white background. Observe for the appearance of blue-purple colour within 10-30 seconds.

Quality control

Test Organisms (ATCC)	DMACA
Escherichia coli (25922)	+
Pseudomonas aeruginosa (27853)	–
Klebsiella pneumoniae (13883)	–

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)

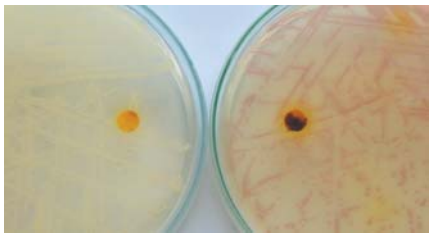
(3) G.D. Fay, A.L. Barry, Methods for Detecting Indole Production by Gram-negative Non-Spore Forming Anaerobes., Appl. Micro. 27, 562 (1974)

(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)



Picture ..... DMACA Indole Disks

1. *Staphylococcus aureus*
2. *Escherichia coli*

Fluka Cat. No. 40405 Hippurate Disks ..... The hippurate disks are recommended for qualitative processes of detection of organisms that have hippurate hydrolase.

The hippurate disks are recommended for qualitative detection of organisms that have hippurate hydrolase. Hippurate hydrolase promotes 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 nynhydrin. However, it must be taken into account that any free amino acid will generate false positive results. The disk method is a rapid test for the presumptive identification of *Gardnerella vaginalis, Campylobacter jejuni, Listeria monocytogenes* and β-hemolytic group B *Streptococci*.

Composition

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

Instructions

Aseptically place the hippurate disk in the brain heart infusion broth (Fluka Cat. No. 53286) inoculated with a suspect colony, incubate at 35°C for 48 hours and 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 flocculants precipitate persists after shaking during 10 minutes, then 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. Put 75 mL of distilled water in a 100 mL graduated flask. Pipette cautiously 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 colour.

Quality control

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

Test Organisms (ATCC)	Growth	Hippurate hydrolysis
Enterococcus faecalis (29212)	+++	–
Streptococcus agalactiae (4768)	+++	+
Streptococcus pyrogenes (19615)	+++	–

\+ = Brown flocculants precipitate persisting after shaking during 10 minutes.

– = If any visible precipitate 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) 2. R.R. Facklam, et al., Presumptive identification of group A, B, and D *Streptococci*., Appl. Microbiol., 27(1), 107 (1974)

(3) 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)

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(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)

Fluka Cat. No. 01869 Hippurate Strips Kit ..... The hippurate strips kit is recommended for detection of organisms that have hippurate hydrolase.

Test for identification of hippurate hydrolase activity of group *B. streptococci, Campylobacter jejuni, Gardnerella vaginalis,* and other microorganisms.
The test is based on hydrolysis of substrate (sodium hippurate) by the bacterial enzyme hippurate hydrolase, and production of benzoic acid and glycine. Glycine produced by this enzymatic reaction is detected after 24 hours of incubation in reaction with chromogen (ninhydrine) and is indicated by the presence of a blue-purple color.

Composition:

The kit contains 50 hippurate strips (Fluka 92472) saturated with sodium hippurate and 350 mg chromogen in 15 ml diluent (Fluka 01875)

Storage:

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

Directions:

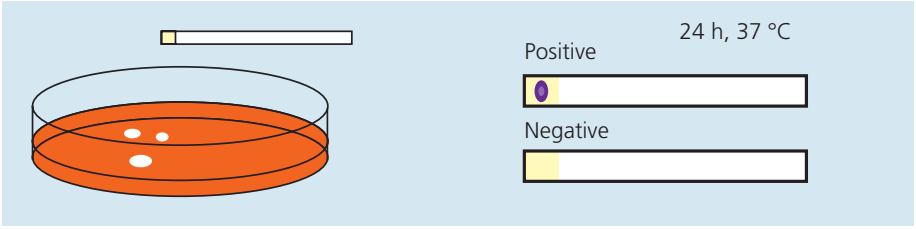
1. Prepare bacterial suspension from pure 24-hours-old culture of tested microorganisms in 0.5 ml of saline solution. (Use a narrow tube so that the entire paper zone of the strip can be exposed to the suspension. The density of the suspension can reach up to 2 ° on the McFarland turbidity scale).
2. Put substrate strip into tube with suspension so that the entire paper 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 wall of the tube. Do not mix.
5. Incubate at laboratory temperature (18–24 °C) for 5–10 minutes.
6. Read evaluate results of test.

Interpretation of results:

Negative reaction: no color change occurs
Positive reaction: a blue-purple color develops in the place of contact between the reagent and inoculum; a so-called "ring" of blue-purple appears

The list below illustrates control strains in routine use:

Test Organisms (ATCC)	Result
Enterococcus faecalis (29212)	negative
Streptococcus agalactiae (4768)	positive
Bacillus subtilis (31193)	positive
Streptococcus pyrogenes (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.

Fluka Cat. No. 06728 Hydrogen Sulfide Test Strips (Lead Acetate Test Strips, H_2S Test Strips) ..... Hydrogen sulfide test strips are used for detection of H_2S production by microorganisms.

A large number of bacteria can produce H_2S in small amounts from sulfur containing amino acids in carbohydrate media. When combined with lead acetate, the H_2S will produce a black precipitate, giving rise to a visible black coloured reaction on the paper strip. The lead acetate method is very sensitive, allowing the detection of trace levels of hydrogen sulphide.

Composition

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

Instructions

Inoculate peptone water (Fluka 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. In the case of negative response, no blackening should appear.

Test Organisms (ATCC)	H_2S production
Escherichia coli (25922)	–
Salmonella serotype Enteritidis (13076)	+
Salmonella serotype Typhimurium (14028)	+

References

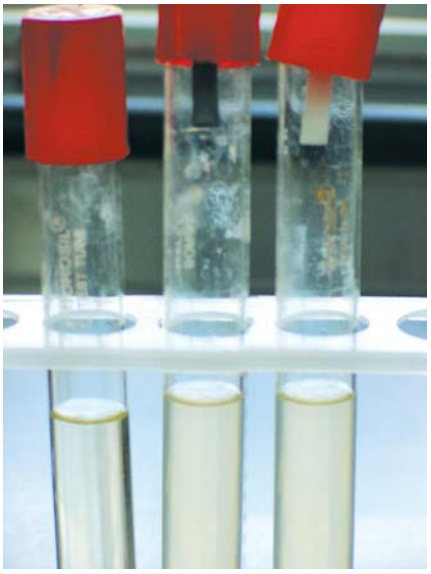
(1) R.N. Collins, M.D. Treger, J.B. Goldsby, J.R. III Boring, D.B. Coohon, R.N. Barr. Interstate outbreak of *Salmonella newbrunswick* infection traced to powdered milk., JAMA, 203, 838 (1968)

(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)



Picture ..... Hydrogen Sulfide Test Strips

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

Fluka Cat. No. 04739 Indoxyl Strips (Acetoxyindol Strips) ..... The indoxyls strips are a diagnostic test for the rapid detection of the acetate esterase activity of microorganisms.

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

Composition

Each package contains 100 test plastic strips with an active zone saturated with 3-acetoxy indol (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 grown for 18-24 hours in a Petri dish, using the paper 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 strip can be moisturised by using condensed water from a lid of the dish or by adding approximately 10 µl of distilled water. When wiping the colonies with a microbiological loop, please ensure that you do not use one of metal construction.

Interpretation of results

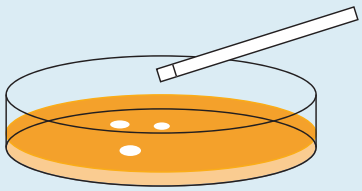
Negative reaction

No colour change develops at the position of wiped colony.

Positive reaction

A blue-green spot develops at the position of wiped colony.

Test Organisms (ATCC)	Result
Escherichia coli (25922)	negative
Branhamella catarrhalis (25238)	positive



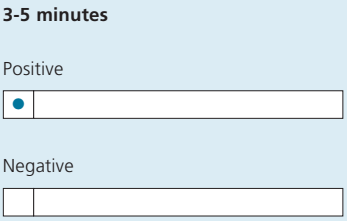


Figure ..... Indoxyl Strips

Quality control

The table above illustrates the perfomance 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)

Fluka Cat. No. 78719 Kovac's Reagent Strips (Indole Reagent Test strips according to Kovac) ..... The Kovac's reagent strips are used for determination of the ability of microorganisms, primarily Enterobacteriaceae , to use tryptophanase.

Tryptophanase, present in species such as *Escherichia coli*, cleaves tryptophan to indole and α-aminopropionic acid. The p-aminobenzaldehyde present in the reagent reacts with the indole to form a pink complex.

Composition

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 and then slowly adding 50 mL of concentrated hydrochloric acid.

Instructions

Indole production by the organism is observed by inserting the Kovac's reagent strips between the plug and inner wall of the tube, above the inoculated peptone water (Fluka Cat. No. 70179) and incubating at 35°C for 18-24 hours.

Quality control

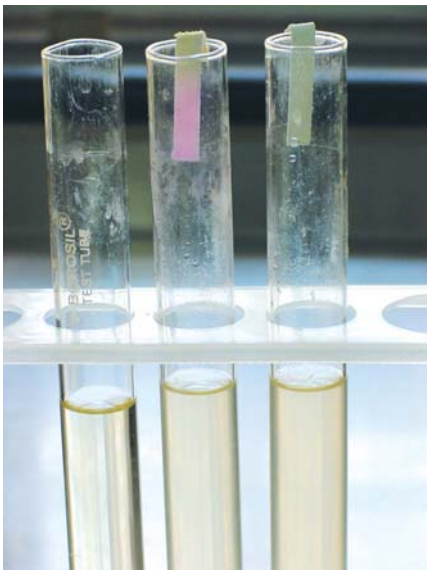
Culture response appears after incutabion in peptone water (Fluka Cat. No. 70179) for 18-24 hours at 35°C. Pink coloration on the lower part of the strip should be considered a positive reaction, as the microorganisms have tryptophanase activity. For negative reactions there is no colour change.

References

(1) J.F. MacFaddin, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore (1980)

(2) A.E. Greenberg, R.R. Trussell, L.S. Clesceri (Eds.), Standard Methods for the Examination of Water and Wastewater, 16th ed., A.P.H.A, Washington, D.C. (1985)

Test Organisms (ATCC)	Indole production
Escherichia coli (25922)	+
Enterobacter aeogenes (13048)	–



Picture ..... Kovac's Reagent Strips

1. Control
2. *Escherichia coli*
3. *Staphylococcus aureus*

Fluka Cat. No. 56348 β-Lactamase Strips ..... The β-Lactamase strips are suitable for the rapid acidimetric detection of β-lactamase activity in microorganisms.

The β-lactamase test is based on hydrolysis of the β-lactam ring in benzylpenicillin, which results in the production of penicilloic acid. This process causes acidification of the bacterial suspension, therefore changing the colour of the acid-base indicator. The acid-base test for β-lactamase activity is suitable only for the detection of *Haemophilus influenzae, Neisseria gonorrhoeae* and *Staphylococcus spp*. This test is not suitable for the detection of the β-lactamase activity in microorganisms such as *Branhamella catarrhalis, Enteroccocus faecalis, Neisseria meningitis, Enterococcus spp*. and others.

Composition

Each package contains 100 test strips with an active zone saturated with benzylpenicillin 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.

Instructions

Solid Media Test

Wipe off several suspect colonies from a Petri dish by the function zone of a diagnostic strip. Mark the strip and incubate at room temperature. The results can be read after 2-10 minutes. 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 present, then the active zone of the strip can be moisturised by using condensed water condensed on the lid of the dish or by adding approximately 10 µl of distilled water.

Liquid Media Test

Prepare approximately 0.5-1 mL of bacterial suspension in a saline solution (2-4 loops). Insert the test strip in the test tube with the prepared bacterial suspension, shake and incubate at room temperature. Read result after 2-10 minutes.

Interpretation of results

Negative reaction

Solution remains red or no colour change develops at the position of wiped colony.

Positive reaction

Solution turns yellow or a blue-green spot develops at the position of wiped colony.

References

(1) R. Bonnet, C. Chanal, E. Ageron et al., Inducible AmpC β-Lactamase of a New Member *Enterobacteriaceae*, Antimicrob. Agents Chemother., 46, 3316 (2002)

(2) L. Shan, et al., Kinetic Analysis of an Inhibitor-Resistant Variant of the OHIO-1 β-lactamase, an SHV-Family Class A Enzyme, Biochem. J., 333, 395, Printed in Great Britain (1998)

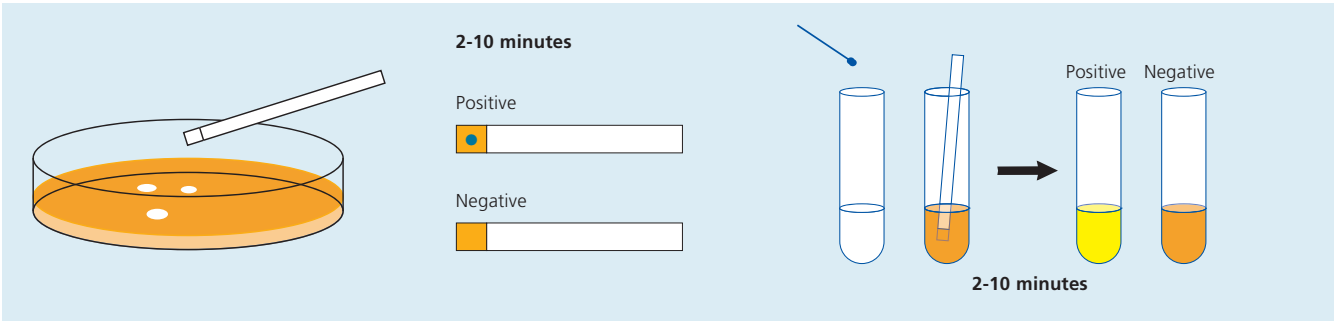


Figure ..... ß-Lactamase Strips

Fluka Cat No. 51138 Nitrate Reagent Disks Kit ..... The nitrate reagent disks are used to detect the reduction of nitrate.

The nitrate test involves the detection of the enzyme nitrate reductase, that causes the reduction of nitrate to nitrite in the presence of a suitable electron donor. Nitrite can be tested by using an appropriate coulorimetric reagent.

Composition

Each package contains 50 sterile filter paper discs (diameter 6mm) impregnated with Nitrate reagent (Fluka 08086) and 1 vial Rehydrating fluids (Fluka 43505) à ca. 5 ml.

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 suitable agar medium plate containing the nitrate substrate. Place the nitrate reagent disks on suspected colony. For enhanced color intensity add a drop of the Rehydrating fluid provided in the kit in the disk.

Principle

Reduction of nitrate (NO_3) to nitrite (NO_2) and to nitrogen gas (N_2) usually takes place under anaerobic conditions (1). Most facultative anaerobes can reduce nitrate in the absence of oxygen and almost all Enterobacteriacea 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 (2). 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 (2). Depending upon the environmental conditions, these products are usually not further oxidised or assimilated into cellular metabolism, but are excreted into the surrounding medium.

Interpretation of results

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

Quality control

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

Test Organisms (ATCC)	Growth	Nitrate reduction
Acinetobacter calcoaceticus (19606)	+++	–
Enterobacter aerogenes (13048)	+++	+
Escherichia coli (25922)	+++	+
Salmonella serotype Typhimurium (14028)	+++	+

References

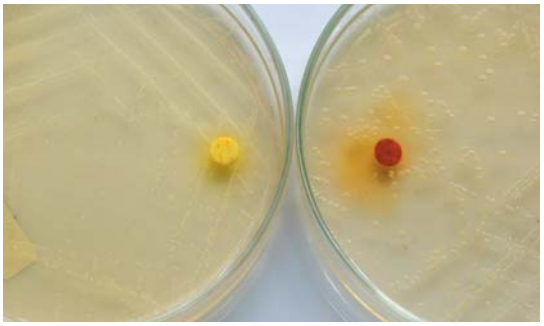
(1) Jr. M.J. Pelczar, R.D. Reid, Microbiology, 2nd ed., McGraw-Hill, New York, 567 (1965)

(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., Finegold S.M., Bailey and Scott's Diagnostic Microbiology, 8th ed., St Louis: Mosby, (1990)



Picture ..... Nitrate Reagent Disks

1. *Acinetobacter calcoaceticus*
2. *Salmonella typhimurium*

Fluka Cat. No. 49862 Nitrocefin Disks ..... For the rapid detection of β-lactamase enzymes in isolated colonies of *Neisseria gonorrhoeae, Moraxella catarrhalis, Staphylococcus spp., Haemophilus influenzae* and anaerobic bacteria.

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

Storage
Store in the freezer below –10°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 deionised 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 deionised 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 colour.

Interpretation of results
Positive
Development of a red colour in the area of the disk where the culture was applied. Note the colour change does not normally develop over the whole of the disk.

Negative
No colour 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 guarantee susceptibility.

Quality control
The table below illustrates the perfomance 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
Haemophilus influenzae (35036)	negative
Neisseria gonorrhoeae (31426)	positive
Staphylococcus aureus (11632)	positive
Escherichia coli (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 results 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 β-lactamase is enhanced by testing growth from a medium containing sub-inhibitory concentrations of a β-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., Yolken R., 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 β-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 β-Lactamase Production., Microbios 20:39-46 (1977)

Fluka Cat. No. 49940 ONPG Disks (2-Nitrophenyl β-D-galactopyranoside Disks, β-Galactosidase Test Disks) ..... ONPG disks are used to detect the presence of β-galactosidase, an enzyme found in lactose-fermenting organisms.

Lactose utilization depends upon two enzymes: a β-galactoside permease, not present in late lactose fermenters, which catalyzes transport of lactose into the cell and β-galactosidase, which breaks down lactose into galactose and glucose. β-Galactosidase is not lactose specific and can act on simple galactosides including the ONPG (o-nitrophenyl-β-D-galactopyranose) substrate. ONPG hydrolysis results in the release of galactose, and o-nitrophenol, an 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. To group Enterobacteriaceae the ability of fermenting lactose is routinely used.

Composition

Each package contains 50, 6 mm diameter sterile filter paper disks impregnated with o-nitrophenyl-β-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 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 during 4 hours at 35°C.

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)

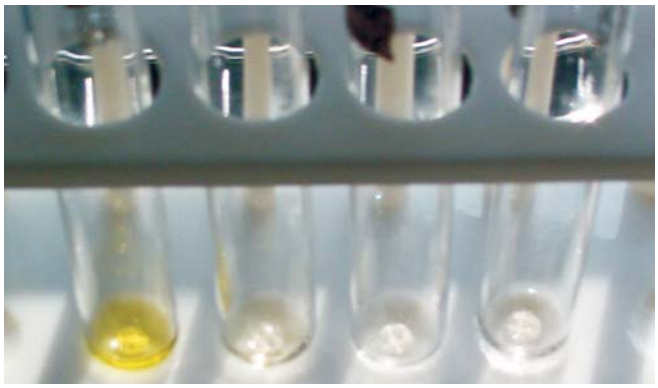
(3) V.E. Becker, H.J. Evans, The influence of Monovalent Cations and Hydrostatic Pressure on β-Galactosidase Activity., Biochim. Biophys. Acta, 191, 95 (1969)

(4) M.C. Neville, G.N. Ling, Synergistic Activation of β-Galactosidase by Na^+ and Cs^+., Arch. Biochem. Biophys., 118, 596, (1967)

(5) J. Lederberg, The β-Galactosidase of *Escherichia coli*, strain K-12., J. Bact., 60, 381 (1950)

Picture ..... ONPG Disks (from left to right)

1. Positive Colony
2. Negative Colony
3. Negative Colony
4. Negative Control



Test Organisms (ATCC)	ONPG Hydrolysis
Citrobacter freundii (8090)	+
Enterobacter aerogenes (13048)	+
Escherichia coli (25922)	+
Proteus vulgaris (8427)	–
Salmonella arizonae (13314)	+
Salmonella serotype Typhimurium (14028)	–

Fluka Cat. No. 74042 Optochin Disks ..... The optochin test is a useful diagnostic tool for identification / differentiation of 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 α-haemolytic (viridans) Streptococci and Pneumococci *(Streptococcus pneumoniae*) cannot be easily differentiated on a blood agar plate (show both a partial clearing of blood and a greenish discolouration; α-haemolysis). 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 (1).

Composition

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

Instructions

Gram staining test (Fluka Cat. No. 77730) is required, followed by the optochin sensitivity test. Prepare tryptone soya agar (Fluka Cat. No. 22091) with blood or blood agar (Fluka Cat. No. 70133) plates and streak pure culture of 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 centre 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 (Fluka Cat. No. 22091) with blood using optochin disks.

Test Organisms (ATCC)	Diameter of zone of inhibition
Streptococcus pneumoniae (6303)	15 mm
Streptococcus pyogenes (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)

Fluka Cat. No. 40560 Oxidase Strips ..... The oxidase strips test is a diagnostic test for the detection of the cytochrome oxidase activity in microorganisms within 1 minute.

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

Composition

The kit contains 100 plastic strips with a paper zone saturated with a solution of N,N-dimethyl-1,4-phenylenediamine and α-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 by the paper zone of diagnostic strip. Read result after 1 minute.

For accurate results, store dry. The aluminum tube containing the strips cannot 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 moisturised by using water condensed on to the lid of the dish or by adding approximately 10 µl of distilled water. When wiping the colonies with a microbiological loop, please ensure that you do not use one of metal construction.

Principle and Interpretation of Results

Gordon and McLeod (1) 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 α-naphthol. Later, Gaby and Hadley (2) introduced a more sensitive method by using N,N-dimethyl-p-phenylenediamine oxalate, establishing that Staphyloccoci are Gram-negative.The cytochrome oxidase present in most Gram-negative bacteria triggers the reaction of N,N-dimethyl-p-phenylenediamine with α-naphthol, forming indophenol blue.

Oxidase test is mainly used to differentiate

1. Oxidase positive *Neisseria* from other Gram-negative diplococci
2. Oxidas 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 false negative reaction may be given by *Vibrio*, *Aeromonas*, and *Plesimonas spp* when grown on a medium containing fermentable carbohydrate such as MacConkey Agar (Fluka Cat. No. 70143). Colonies taken from media containing nitrate may give unreliable results. The loss of activity of the oxidase reagent is caused by auto oxidation which may be avoided by adding 0.1% ascorbic acid (Fluka Cat. No. 95209).

Negative reaction

No colour change develops at the position of wiped colony.

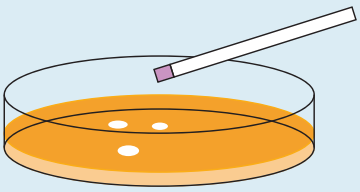
Positive reaction

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

Quality control

The list below illustrates control strains in routine use.

Test Organisms (ATCC)	Result
Escherichia coli (25922)	negative
Pseudomonas aeruginosa (27853)	positive



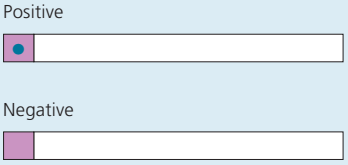


Figure ..... Oxidase Strips

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)

Fluka Cat. No. 70439 Oxidase Test Disks ..... The oxidase test is an important differential procedure which should be performed on all Gram-negative bacteria that are to be identified.

Composition

Each package contains 50 disks impregnated with N,N-dimethyl-p-phenylenediamine oxalate and α-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 a inoculating loop or a toothpick, touch and spread a well isolated colony on a oxidase disk. The reaction is observed within 2 minutes at 25-30°C.

Principle and Interpretation of Results

Gordon and McLeod (1) 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 α-naphthol. Later, Gaby and Hadley (2) introduced a more sensitive method by using N,N-dimethyl-p-phenylenediamine oxalate, establishing that *Staphyloccoci* are Gram-negative.The cytochrome oxidase present in most Gram-negative bacteria triggers the reaction of N,N-dimethyl-p-phenylenediamine with α-naphthol, forming indophenol blue.

Oxidase test is mainly used to differentiate

1. Oxidase positive *Neisseria* from other Gram-negative diplococci.
2. Oxidas 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 false negative reaction may be given by *Vibrio*, *Aeromonas*, and *Plesimonas* species when grown on a medium containing fermentable carbohydrates such as MacConkey Agar (Fluka Cat. No. 70143). Colonies taken from media containing nitrate may give unreliable results. The loss of activity of the oxidase reagent is caused by auto oxidation which may be avoided by adding 0.1% ascorbic acid (Fluka Cat. No. 95209).

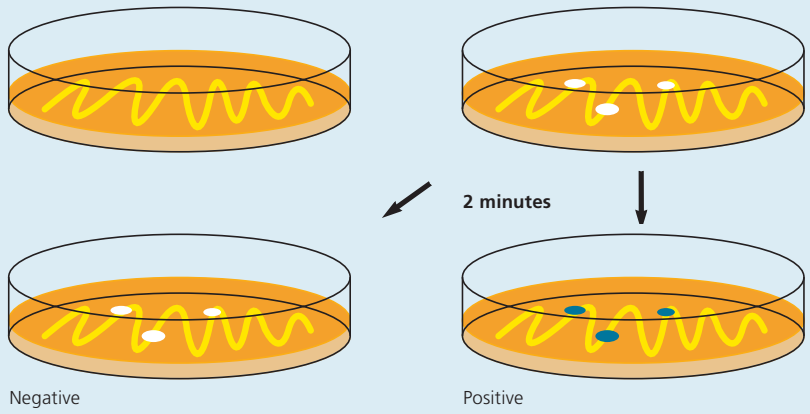
Reaction takes place within 2 minutes at 25-35°C.

Organisms (ATCC)	Reaction	Colour
Pseudomonas aeruginosa (27853)	positive	deep purple blue
Staphylococcus aureus (25923)	negative	–
Neisseria gonorrhoeae (19424)	positive	deep purple blue
Escherichia coli (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)

Picture ..... Oxidase Disks



Fluka Cat. No. 67886 PYRase Strips (Pyrrolidonyl Peptidase Strips) .....

Diagnostic test for the rapid differentiation of *Enterococci* from the group D Streptococci and differentiation of *Streptococcus pyrogenes* from other haemolytic Streptococci.

Composition

The kit contains 50 plastic strips with a paper 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 by the paper zone of diagnostic strip. After one minute, apply approximately 10 µl of developing reagent to the paper zone using a plastic loop or micropipette. The reaction is observed within 1 minute.

Interpretation of results

Negative reaction

No colour change develops at the position of wiped colony.

Positive reaction

A red spot develops at the position of wiped colony.

Use PYRase test due to the recommendation of NRL for Streptococci and Enterococci.

Positive (change to red colour)	Negative (no colour change)
Enterococcus	*Streptococcus bovis*
Streptococcus pyrogenes	*Streptococcus equinus*
Lactococcus lactis	Beta Minute haemolytic *Streptococci*:
Aerococcus	*Streptococcus anginosus*
Gamella (most strains)	*Streptococcus intermedius*
	Streptococcus constellatus
	Other *Lactococcus* species
	Leuconostoc
	Pediococcus
	Lactobacillus (sporadic positivie test)
	Other species of *viridans Streptococci*

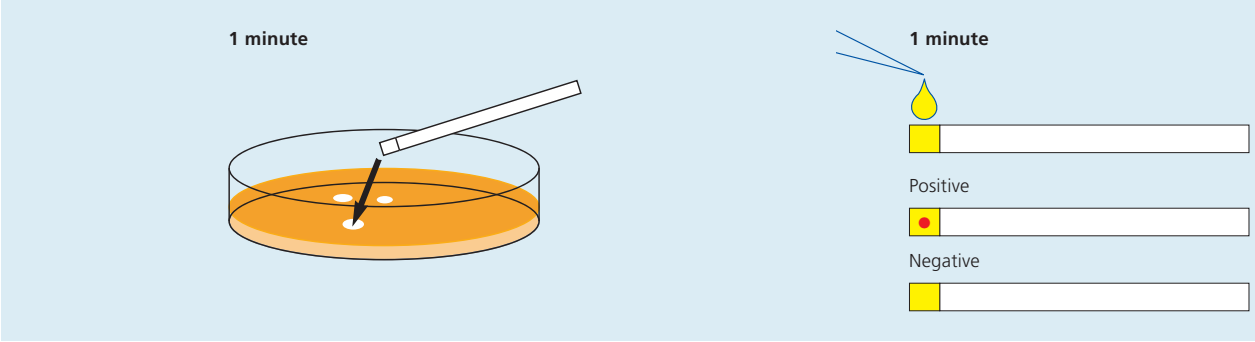


Figure ..... PYRase Strips

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(1) Godsey et al., Abstr. ASM Annu. Meet., C84, 276 (1981)

(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. Wasilauskas, 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. Yolken, 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)

Fluka Cat. No. 74041 Sterility Indicator (Steam Sterilization) ..... These indicators consist of ca. 1 million *Bacillus stearothermophilus* (ATCC 7953) spores impregnated on paper strips, individually placed into envelopes.

Bacillus stearothermophilus is a thermophilic species that can grow at temperatures such as 65°C and higher. Its spores are an excellent tool to monitor autoclave performance, as they are highly resistant to temperature. These indicators are similiar to those specified by the United States military specification MIL-S-36586 are GMP requirements of the United States FDA.

Composition

Each package contains 25 test strips. One paper strip is impregnated with 1 million spores of *Bacillus stearothermophilus* (ATCC 7953).

Instructions

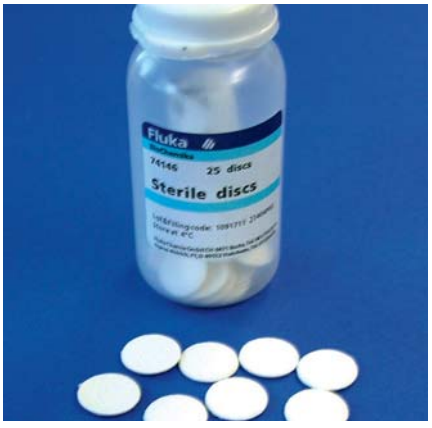
Place the indicators in such a manner that they are exposed to the steam in representative places of the media and the equipment. For routine evaluation of each radiated lot, a standard procedure should be established. After steam sterilization open the envelope with the strip inside by using strict aseptic techniques. Inoculate the strip after autoclaving in a tube with sterile CASO broth (Fluka Cat. No. 22098) and incubate at 55-60°C up to 7 days. Also, 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 (Fluka Cat. No. 22098):

Spore Strip	Result
Exposed to steam	no growth
Unexposed Luxuriant	growth

Fluka Cat. No 74146 Sterile Disks ..... The sterile disks can be used to test a variety of antibiotics, carbohydrates, substrates, 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 a solution or apply some solution on the disks. Allow to dry and place it in an inoculated agar plate. Each disk will absorb exactly the same amount of liquid.

Picture ..... Sterile Disks

Fluka Cat. No. 05290 Sterility Indicator (Radiation Sterilization) .....

These indicators consists of ca. 1 million *Bacillus pumilus* (ATCC 27142) spores impregnated on paper strips, individually placed into envelopes.

Bacillus pumilus was chosen due to its resistance to radiation. Its spores are an excellent tool to monitor the efficiency of radiation sterilization since they are highly resistant. The above mentioned indicators are similar to those specified by the U.S. military specification MIL-S-36586 and are GMP requirements of the United States FDA.

Composition

Each package contains 25 test strips. One paper strip is impregnated with 1 million spores from *Bacillus pumilus* (ATCC 27142).

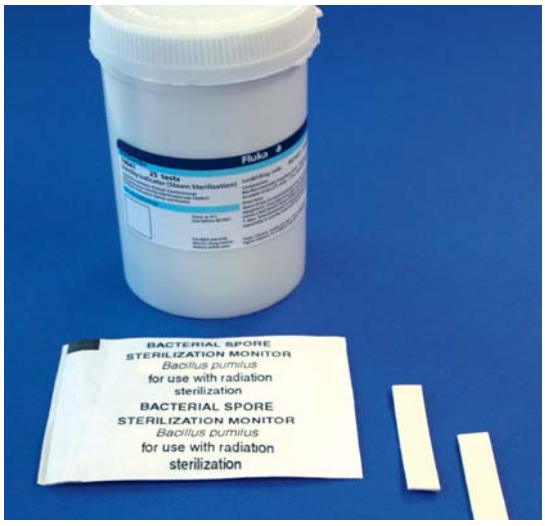
Instructions

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 radiation sterilization, open the envelope with the strip using rigid aseptic techniques. Inoculate strip in a tube with sterile CASO broth (Fluka Cat. No. 22098) and incubate at 35-37°C up to 7 days. Also, 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 (Fluka Cat. No. 22098):

Spore Strip	Result
Exposed to radiation	No growth
Unexposed	Luxuriant growth



Picture ..... Sterility Indicator

Fluka Cat. No. 75744 Tributyrin-Strips (TRIBU Strips) ..... Tributyrin strips are a diagnostic test for the differentiation of *Branhamella* and *Neisseria*. The test principle is the enzymatic hydrolisis of tributyrin, a reaction that causes a colour change of acid-base indicator. The results can be read after 18-20 hours.

Composition

Each package contains 300 test strips saturated with tributyrin and 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 sterilized. Observe the maintenance of sterility when the strips are used repeatedly. To obtain accurate results, avoid moisture during storage. Allow to equilibrate to room temperature before opening the container. When 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 1mL suspension of the test strain in buffered saline (pH 7.2). Incubate the test sample at 37°C (without CO_2). Preliminary results can be read after several hours when the red colour changes to yellow (positive result). The final result can be obtained after 18-20 hours incubation.

Interpretation of results

Negative reaction

Red colour did not change to yellow (*Neisseria*)

Positive reaction

Red colour change to yellow (*Branhamella*)

Identification diagram for *Branhamella*

Tributyrin Reduction of nitrates	
+ ***Branhamella***	– ***Neisseria***
saccharides +/– β-lactamase +/–	saccharides +/–
+ adapted parasite	– opportune pathogen

Quality control

The list below illustrates control strains in routine use.

Test Organisms (ATCC)	Result
Neisseria gonorrhoeae (19424)	negative
Branhamella catarrhalis (25238)	positive

References

(1) Berger U., Über die Spaltung von tributyrin durch *Neisseria*., Arch Hyg. Bakteriol., 146:388-391 (1962)

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(8) Forbes B.A., et al. Bailey and Scott's Diagnostic Microbiology, 10th ed. C.V. Mosby Company, St. Louis, MO, (1998)

Picture ..... Tributyrin-Strips reactions

Fluka Cat. No. 77148 Differentiation Disks X Factor / Fluka Cat. No. 89788 V Factor / Fluka Cat. No. 08482 X + V Factors ..... Use for the presumptive identification of *Haemophilus* species on the basis of their requirements 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 growth. The need for either one or both factors provides a way for differentiation of these organisms.

Composition

Each package contains 50 test disks. Sterile 6mm diameter filter paper disks impregnated with:

- hemin and nicotinamide adenine dinucleotide (X + V factor disks; Fluka Cat. No. 08482).
- hemin (X factor disks; Fluka Cat. No. 77148).
- nicotinamide adenine dinucleotide (V factor disks; Fluka Cat. No. 89788).

Instructions

Inoculate the surface of a blood agar (Fluka Cat. No. 70133) or brain heart infusion agar (Fluka Cat. No. 70138) plate 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	Place
X factor disk	12 o'clock
V factor disk	4 o'clock
X + V factor disk	8 o'clock

Observe the growth in the neighbourhood of the disk. The test organism requiring X factor grows only in the vicinities of X disks. Those who require V factor grows only in the vicinities of V disks. The ones who require V and X factor grows only in the vicinities of X + V disks.

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

Quality control

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

Test Organisms (ATCC)	Without growth factor	X factor	Y factor	X +Y factor
Haemophilus influenzae (35056)	–	–	–	+
Haemophilus parainfluenza (7901)	–	–	+	+
Haemophilus ducreyi (27722)	–	+	–	+
Bordetella petussi (13048)	+	+	+	+

References

(1) D.J. Davis, J. Infect. Dis., 21, 392 (1917)
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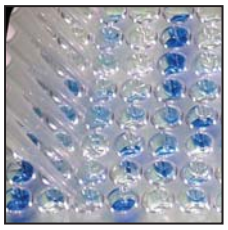

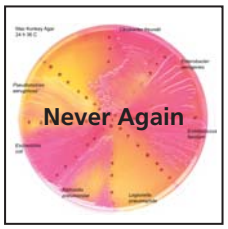

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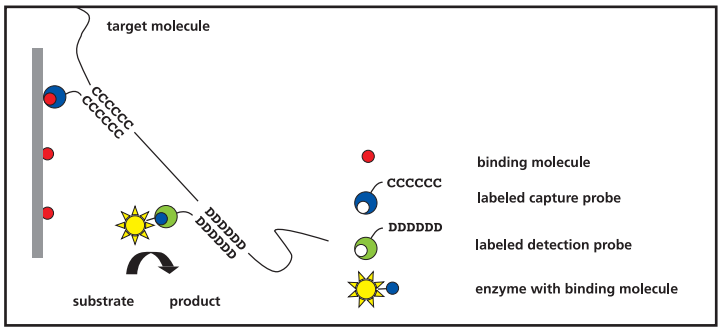
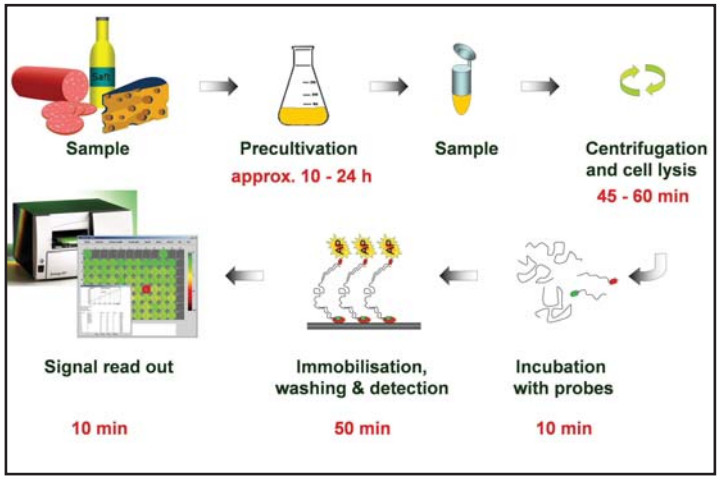


How it works:



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