Diatabs™ User's Guide

Diagnostic Tablets for Bacterial Identification

2024

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Diatabs™- DIAGNOSTIC TABLETS for BACTERIAL IDENTIFICATION

The **Diatabs™**, Diagnostic Tablets (Diatabs™), developed by Rosco Diagnostica are identification tests made available as individual tablets, which allow the microbiologist a free choice of the most appropriate tests for identification. Most of the Diatabs™ are rapid tests (chromogenic enzymatic reactions, and modified conventional tests). The tablets may be used as single tests to show isolated microbial properties or as part of cost-effective systems of their own.

This User's Guide describes more than 80 different types of tests for identification of the clinically important groups of bacteria and has been written by J.B. Casals on behalf of Rosco Diagnostica. The 9th Ed. 2024 of the Diatabs™ User's Guide contains updated text, tables and references, all necessary information when using Diatabs™ tablets for identification of bacteria and yeasts.

Finally, we would like to quote The Manual of Clinical Microbiology 8th Ed. 2003, page 893:

"Individually available tablets … (Rosco Diagnostic Tablets …) are much cheaper than commercial kits, they can be applied in a number of situations and allow flexibility in tailoring the set to best suit special needs".

The User's Guide is available at our website www.rosco-diagnostica.com and updated information is continuously included.

ROSCO Diagnostica ApS is welcoming any feedback and questions on bacterial identification from users directly (info@rosco-diagnostica.com) or through our representatives.

ROSCO DIAGNOSTICA ApS

Bacterial identification using Diatabs™

Rosco Item No.	Diatabs™	Use	Document no.
55711	Acetamide Hydrolysis (50)	Non-Fermenters	**3.1.0**
52011	Adonitol (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
55911	Alkaline Phosphatase (50)	Staphylococci/Anaerobes/Gemella	**3.2.0**
50111	Alpha-Fucosidase (50)	Anaerobes/Streptococci	**3.20.2**
50211	Alpha-Galactosidase (50)	Non-fermenters/Streptococci/Anaerobes	**3.20.4**
50411	Alpha-Glucosidase (50)	Non-fermenters/Anaerobes/Gardnerella	**3.20.6**
50711	Alpha Mannosidase (50)	*Listeria* spp. /Arcanobacterium/Actinomyces	**3.20.8**
52111	l-Arabinose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
56211	Arginine Dihydrolase (ADH) (50)	Staphylococci/Streptococci/Non-fermenters/ Vibrionaceae/Lactic bacteria (Vanco R)	**3.5.0**
40211	Bacitracin Low (50)	Group A-streptococci/Gardnerella	**3.6.0**
70812	Bacitracin 40 U Neo-Sensitabs™ (50)	Screening *Haemophilus* spp.	**3.7.0**
50011	Beta-N-Acetylglucosaminidase (50)	Anaerobes/Streptococci/Actinomyces	**3.20.1**
59911	Beta-Fucosidase (50)	Streptococcus anginosus group	**3.20.3**
50311	Beta-Galactosidase (ONPG) (50) Fermenters/	Neisseria/Enterobacteriaceae/Non- Anaerobes/Actinobacillus/Pasteurella	**3.20.5**
50511	Beta-Glucosidase (50)	Staphylococci/Streptococci	**3.20.6**
50611	Beta-Glucuronidase (PGUA) (50)	*E.coli*/Enterobacteriaceae/Anaerobes/ Streptococci/Arcanobacterium	**3.20.7**
45511	Beta-Lactamase (50)	Haemophilus/Neisseria/Staphylococci	**3.8.0**
50811	Beta-Xylosidase (50)	Enterobacteriaceae/Capnocytophaga/ Acinetobacter	**3.20.9**
40411	Bile Esculin (50)	Enterococci, Lactic bacteria (Vanco R)	**3.10.0**
10411	Phenylboronic Acid (50)	Detection of AmpC	**3.9.0**
40511	Brilliant Green (50)	Anaerobes	**3.4.0**
41611	C-390 40 µg (50)	*Pseudomonas aeruginosa*	**3.11.0**
	Cellobiose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
56511	Citrate (50)	Enterobacteriaceae/Non-Fermenters	**3.12.0**
10311	Cloxacillin 500 µg (50)	Detection of AmpC	**3.9.0**
41811	Colistin 10 µg (50)	Anaerobes/Neisseria/Non-Fermenters	**3.4.0**

58911	Cycloheximide (50)	*Candida* spp.	**3.13.0**
59611	Deferoxamine 250 µg (50)	*Staph. epidermidis/Staph. hominis*, Non-Fermenters	**3.14.0**
	Dipicolinic Acid	Detection of MBL	**3.9.0**
	Dulcitol (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
56611	Esculin Hydrolysis (50)	Streptococci/Enterococci/Yersinia	**3.10.0**
42611	Factor V (50)	Haemophilus	**3.17.0**
42511	Factor X (50)	Haemophilus	**3.17.0**
42711	Factor X+V (50)	Haemophilus	**3.17.0**
74212	Fosfomycin Neo-Sensitabs™ (50)	Staphylococci, Corynebacteria	**3.18.0**
	Fructose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
74412	Furazolidone Neo-Sensitabs™ (50)	Staphylococci/Micrococci/Enterococci/ Corynebact.	**3.19.0**
	Galactose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
46711	Gamma-Glutamyl Aminopeptidase (50)	Meningococci/Helicobacter	**3.3.1**
43012	Gentamicin 250 µg Neo-Sensitabs™ (50)	HLR enterococci	**3.16.0**
52611	Glucose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
56711	Hippurate Hydrolysis (50)	Campylobacter/Gardnerella/Streptococci/ Facklamia/Abiotrophia	**3.21.0**
59511	Indoxyl Acetate (50)	Campylobacter/Helicobacter	**3.22.0**
	Inositol (50)		**3.36.0**
52711	Inulin (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
43112	Kanamycin 500 µg Neo-Sensitabs™ (50)	Anaerobes/HLR enterococci	**3.16.0**
52811	Lactose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
58411	LDC/Indole (50)	Enterobacteriaceae/Salmonella ID	**3.15.1**
46811	Leucine Aminopeptidase (50)	Campylobacter/Corynebacteria/ CAT neg. Gram+cocci	**3.3.2**
56811	Lysine Decarboxylase (LDC) (50)	Enterobacteriaceae/Vibrionaceae/ Corynebacteria	**3.23.0**
52911	Maltose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53011	Mannitol (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53111	Mannose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**

53211	Melibiose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
59711	Metronidazole 5 µg (50)	Anaerobes	**3.24.0**
43611	Metronidazole 50 µg (50)	Gardnerella	**3.25.0**
75712	Mupirocin 10 µg Neo-Sensitabs™ (50)	Staphylococci/Micrococci/Enterococci/ Corynebact.	**3.19.0**
43711	Nitrate Reduction (50)	Staphylococci/Non-Fermenters, Anaerobes	**3.26.0**
46312	Novobiocin 5 µg Neo-Sensitabs™ (50)	Staphylococci/Peptostrep./Pediococci	**3.27.0**
45411	O/129 (Vibriostaticum) 150 µg (50)	Vibrionaceae, Corynebacteria	**3.28.0**
59111	ODC/Indole (50)	Enterobacteriaceae/Citrobacter spp.	**3.15.2**
50311	ONPG (Beta Galactosidase) (50)	Neisseria/Enterobacteriaceae/Non-Fermenters/ Anaerobes/Actinobacillus/Pasteurella	**3.20.5**
44211	Optochin (50)	Pneumococci	**3.29.0**
57011	Ornithine Decarboxylase (ODC) (50)	*Staph. lugdunensis*/Enterobacteriaceae/ Haemophilus/Corynebacteria	**3.23.0**
44311	Oxgall (Bile) (50)	Anaerobes	**3.29.0**
45711	Oxidase (50)	Enterobacteriaceae/Non-Fermenters/Neisseria	**3.30.0**
59011	PGUA/Indole (50)	*E. coli*	**3.15.3**
77512	Polymyxins 150 µg Neo-Sensitabs™ (50)	*Staph. aureus*/Shewanella/Kingella	**3.31.0**
57311	Porphyrin (d-ALA) (50)	Haemophilus/Gram positive cocci	**3.32.0**
46911	Proline Aminopeptidase (50)	Neisseria/Anaerobes/Clostridium difficile	**3.3.0**
59311	Ps. aeruginosa Screen (50)	*Ps. aeruginosa*	**3.33.0**
59811	Pyrazinamidase (50)	Corynebacteria/*Yersinia enterocolitica*	**3.34.0**
47011	Pyrrolidonyl Aminopeptidase (50)	Group A-streptococci/Enterobacteriaceae/ Peptostreptococci/Staphylococci/ Lactic bacteria (Vanco R)	**3.3.4**
53311	Raffinose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53411	l-Rhamnose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
	Ribose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
	Salicin (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53711	Sorbitol (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
44611	S.P.S. (50)	Gardnerella/Peptostreptococci	**3.35.0**
44712	Streptomycin 500 µg Neo-Sensitabs™ (50)	HLR enterococci	**3.16.0**

53811	Sucrose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
57811	TDA or Indole (50)	Enterobacteriaceae/Anaerobes/ Actinobacillus/Pasteurella	**3.37.0**
45011	Tellur (50)	Enterococcus faecalis	**3.38.0**
57411	Tetrathionate Reductase (50)	Enterobacteriaceae/Non-Fermenters	**3.39.0**
53911	Trehalose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
48811	Tributyrin (50)	*Moraxella catarrhalis*/Non-Fermenters/ Corynebacteria	**3.40.0**
47211	Trypsin (50)	Non-Fermenters/Anaerobes/ Capnocytophaga	**3.3.5**
57511	Urease (50)	Enterobacteriaceae/Staphylococci/ Anaerobes/Non-Fermenters	**3.41.0**
57611	Urease/Indole (50)	Enterobacteriaceae/Anaerobes/ Actinobacillus/Pasteurella	**3.15.4**
57911	Urease/TDA (50)	Enterobacteriaceae	**3.15.5**
79312	Vancomycin 5 µg Neo Sensitabs (50)	Anaerobes/Enterococci	**3.4.0**
57711	Voges-Proskauer (50)	Enterobacteriaceae/Streptococci/ Staphylococci	**3.42.0**
54011	d-Xylose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**

The numbers in brackets indicate the number of tablets per vial/cartridge.

AMINOPEPTIDASES:

46711	Gamma-Glutamyl Aminopeptidase (50)	Meningococci/Helicobacter	**3.3.1**
46811	Leucine Aminopeptidase (50)	Campylobacter/Corynebacteria/ CAT neg Gram+cocci	**3.3.2**
46911	Proline Aminopeptidase (50)	Clostridium difficile/Neisseria/ Peptostreptococci	**3.3.3**
47011	Pyrrolidonyl Aminopeptidase (50)	Group A-streptococci/Enterobacteriaceae/ Peptostreptocci/Staphylococci/Enterococci/ Lactic bacteria (Vanco R)	**3.3.4**
47211	Trypsin (50)	Non-Fermenters/Porphyromonas/ Capnocytophaga	**3.3.4**

DOUBLE TEST TABLETS:

58411	LDC/Indole (50)	Enterobacteriaceae	**3.15.1**
59111	ODC/Indole (50)	Enterobacteriaceae/*Citrobacter* spp.	**3.15.2**
59011	PGUA/Indole (50)	*E. coli*	**3.15.3**
57611	Urease/Indole (50)	Enterobacteriaceae/Anaerobes/ Actinobacillus/Pasteurella	**3.15.4**
57911	Urease/TDA (50)	Enterobacteriaceae	**3.15.5**

The numbers in brackets indicate the number of tablets per vial/cartridge.

ESTERASES/LIPASES:

59511	Indoxyl Acetate (50)	Campylobacter/Helicobacter	**3.22.0**
48811	Tributyrin (50)	Moraxella catarrhalis/Non-Fermenters/ Corynebacteria	**3.40.0**

GLYCOSIDASES:

50011	Beta-N-Acetylglucosaminidase (50)	Anaerobes/Streptococci/Actinomyces	**3.20.1**
50111	Alpha-Fucosidase (50)	Streptococci/Prevotella/Porphyromonas	**3.20.2**
59911	Beta-Fucosidase (50)	Streptococcus anginosus group	**3.20.3**
50211	Alpha-Galactosidase (50)	Streptococci/Prevotella/Clostridia	**3.20.4**
50311	ONPG (Beta Galactosidase) (50)	Neisseria/Enterobacteriaceae/ Non-Fermenters/Anaerobes/ Actinobacillus/Pasteurella	**3.20.5**
50411	Alpha-Glucosidase (50)	Non-Fermenters/Gardnerella/Anaerobes	**3.20.6**
50511	Beta-Glucosidase (50)	Staphylococci/Streptococci	**3.20.6**
50611	Beta-Glucuronidase (PGUA) (50)	*E. coli*/Anaerobes/Streptococci/ Arcanobacterium	**3.20.7**
50711	Alpha-Mannosidase (50)	*Listeria* spp./Arcanobacterium/ Actinomyces	**3.20.8**
50811	Beta-Xylosidase (50)	Enterobacteriaceae/Capnocytophaga/ Acinetobacter/Propionibacteria.	**3.20.9**

SUGAR FERMENTATION TESTS:

52011	Adonitol (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
52111	l-Arabinose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
	Cellobiose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**

	Dulcitol (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
	Fructose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
	Galactose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
52611	Glucose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
	Inositol (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
52711	Inulin (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
52811	Lactose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
52911	Maltose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53011	Mannitol (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53111	Mannose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53211	Melibiose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53311	Raffinose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53411	l-Rhamnose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
	Ribose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53611	Salicin (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53711	Sorbitol (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53811	Sucrose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53911	Trehalose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
54011	d-Xylose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**

The numbers in brackets indicate the number of tablets per vial/cartridge.

Storage conditions

1) On receipt check the temperature symbol on the label. Diatabs™ with a 2 °C to 8 °C symbol should be stored in a refrigerator, and Diatabs™ with a 25 °C as an upper temperature symbol on the label should be stored at room temperature.
2) If Diatabs™ are stored in the refrigerator, allow the vials to reach room temperature before opening, i.e. 30-60 minutes, to avoid condensation forming on the tablets.
3) Keep Diatabs™ in vials well protected from direct light and avoid high humidity. Keep, if any, the humidity absorbing material (a desiccant capsule) in the vial.

The expiry date on the vials applies only to vials with lids, stored at the correct temperature.

ACETAMIDE HYDROLYSIS (ACM)

REF No. 55711

Test for demonstration of the ability of bacterial strains to hydrolyse acetamide. Mainly used in differentiation of non-fermenting gram-negative rods.

Procedure

Prepare a dense bacterial suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one Acetamide Hydrolysis Diagnostic Tablet and close the tube.

Incubate at 35-37 °C for 18-24 hours - some positive reactions may be recorded already after 4-6 hours.

Reading of the tests

Positive reaction: **Red**

Negative reaction: Yellow, orange

Results

Acetamide hydrolysis is useful in the differentiation within the **fluorescent** group of Pseudomonas:

	ACM
Pseudomonas aeruginosa	+
Pseudomonas fluorescens	0^{+}
Pseudomonas putida	0

For the differentiation of *Comamonas acidovorans* (+) from *Comamonas testosteroni* (0). Most strains of *Burkholderia cepacia* are positive and most strains of *St. maltophilia* are negative.

Most strains of Alcaligenes (faecalis, denitrificans and *Achr. xylosoxidans*) are positive, while other non-fermenters are negative.

Non-fermenters

ACM positive	ACM negative
Ps. Aeruginosa	*Ps. fluorescens*
Com. acidovorans	*Ps. putida*
Burkh. cepacia	*Com. testosteroni*
Alc. faecalis	*Sten. maltophilia*
Alc. denitrificans	
Achr. xylosoxidans	

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Acetamide hydrolysis (Acetamide)	*Ps. aeruginosa* ATCC 27853	*E. coli* ATCC 25922

References

1) Palleroni, N.J.: Pseudomonas in "Bergey's Manual of Systematic Bacteriology", Vol. 1, 141-199, 1984.

ALKALINE PHOSPHATASE (Alk P)

REF No. 55911

Contain the chromogenic substrate: 4-nitrophenyl phosphatedi (2-amino-2-ethyl-1,3-propanediol) salt that in the presence of alkaline phosphatase releases free 4-nitrophenol (yellow color).

Procedure

Prepare a dense bacterial suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one Alkaline Phosphatase Diagnostic Tablet and close the tube. Incubate at 35-37°C for a **maximum of 4 hours**.

Reading of the tests

Positive reaction: **Strong yellow**

Negative reaction: Colourless or slight yellow

Incubation longer than 4 hours may give a false positive reaction.

Results

1) Staphylococci

Most strains of *S. aureus* and *S. epidermidis* and *S. schleiferi* show a positive reaction, while most strains of *S. hominis*, *S. haemolyticus*, and *S. warneri* show a negative reaction.

	HCF	Alk P (4h)	PYR (1h)	ODC	URE	DEFRX	Poly
S. aureus	100	+	0	0	95	R (≤14 mm)	R (≤12 mm)
S. epidermidis	0	+	0	0+	86	S (≥16 mm)	S
S. haemolyticus	0	0	100	0	0	R	S (≥14mm)
S. hominis	0	0	0+	0	+	S	S
S. lugdunensis	87	0+	100	+	81	R	S
S. schleiferi	100	+	89	0	0	R	S
S. warneri	0	0	V	0	+	R	S

2) Differentiation of *Gemella* spp, *Rothia mucilaginosa and Dolosigranulum pigrum*

(PYR +, LAP +)

	Alk P	SUC	SOR	NO_3	6.5%NaCl	VP	BaciLow	ADH
Gemella bergeriae	0	0	0	0	0	0	R	0
Gemella haemolysans	+	V	0	0	0	V	R	0
Gemella morbillorum	0	+	0^+	·	0	0	R	0
Gemella sanguinis	+	+	+	0	0	V	R	0
Rothia mucilaginosa	0	+	0	+	0	+	S	·
Dolosigranulum pigrum	0	+	0^+	·	+	·	R	$+^0$

Alk P = Alkaline Phosphatase Diatabs™, PYR (1h) = Pyrrolidonyl Aminopeptidase Diatabs™ (1h incubation), ODC = Ornithine Decarboxylase Diatabs™, DEFRX = Deferoxamine Diatabs™, URE = Urease Diatabs™, Poly = Polymyxins 150 µg Neo-S, MAL = Maltose Diatabs™, SUC = Sucrose Diatabs™, SOR = Sorbitol Diatabs™, HCF = Human clumping factor, NO_3 = Nitrate Reduction Diatabs™, VP = Voges Proskauer Diatabs™, BaciLow = Bacitracin Low Diatabs™ (S ≥ 10 mm, R < 10 mm)

3) Useful also in the differentiation of non-fermenters, viridans streptococci and anaerobes.

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Alkaline Phosphatase (p-Nitrophenyl-Phosphate)	*E. coli* ATCC 25922	*S. haemolyticus* ATCC 29970

References

1) Devriese L.A. et al: *Streptococcus hyointestinalis* sp. nov. from the gut of swine. Intl. J. Syst. Bacteriol. **38**, 440-1, 1988.

2) Lindsay J.A., Riley T.V.: Susceptibility to desferrioxamine: a new test for the identification of *Staphylococcus epidermidis*. J. Med. Microbiol. **35**, 45-48, 1991.

3) Collins M.D. et al: Description of *Gemella sanguinis* sp. nov. isolated from human clinical specimens. J. Clin. Microbiol. **36**, 3090-3, 1998.

4) Leung M.J.: Case of *Staph. schleiferi* endocarditis and a simple scheme to identify clumping factor positive staphylococci. J. Clin. Microbiol. **37**, 3353-6, 1999.

AMINOPEPTIDASES

General description

Bacteria may be differentiated on their ability to enzymatically hydrolyze a series of aminopeptidase substrates. The procedure is based upon the enzymatic liberation of beta naphthylamine (beta-NA) from an l-aminoacid- beta-NA substrate. The liberated beta-NA is identified by its reaction with Aminopeptidase reagent producing a red colour in case of positive reactions.

Range

The actual range of aminopeptidases (substrates) comprises:

Arginine Aminopeptidase	(ARG)	(10611)
Gamma-Glutamyl Aminopeptidase	(γ-GLU)	(46711)
Leucine Aminopeptidase	(LAP)	(46811)
Proline Aminopeptidase	(PRO)	(46911)
Pyrrolidonyl Aminopeptidase	(PYR)	(47011)
Trypsin (BAA)	(TRYP)	(47211)

Procedure

Prepare a dense "milky" bacterial suspension (at least McFarland No. 4) from the strain to be tested in 0.25 ml saline in a tube. Add one tablet of aminopeptidase substrate and close the tube. Incubate at 35-37 °C for **4 hours**. In some cases, overnight incubation is required.

After incubation add 3 drops of Aminopeptidase reagent (92231) and read the colour reaction within 5 minutes.

Reading of the tests

	4 h	Overnight
Positive reaction:	**Red/orange**	**Red**
Negative reaction:	Yellow	Yellow/orange

The test may also be read by exposing the tube (no reagent added) to a Wood's lamp (at 360 nm). A blue fluorescence in the supernatant indicates a positive reaction.

General References

1) Peterson E.H., Hsu E.J.: Rapid detection of selected gram-negative bacteria by aminopeptidase profiles. J. Food Sci. **43**, 1853-1856, 1978.

2) Watson R.R.: Substrate specificities of aminopeptidases: a speci fic method for microbial differentiation. Methods Microbiol. **9**, 1-4, 1976.

3) Euzeby J.P.: Activité peptidasique vis a vis des aminoacyl-beta-naphtilamides de quelques especies du genre Bartonella. Dictionaire de Bacteriologie Veterinaire, Sept. 1999.

ARGININE AMINOPEPTIDASE (ARG)

REF No. 10611

The test is based on enzymatic release of beta-naphthylamine from the arginine-beta-naphthylamide substrate. Beta-naphthylamine is detected by its reaction with Aminopeptidase reagent giving a red colour.

Procedure

Prepare a dense bacterial suspension with a turbidity of at least McFarland No. 4 of the strain to be tested in 0.25 ml saline in a tube. Add one arginine aminopeptidase tablet and close the tube. Incubate at 35-37 °C for **4 hours**.

After incubation add 3 drops Aminopeptidase reagent (92231) and read the colour reaction within 5 minutes. See also Aminopeptidase, general description in document **3.3.0**.

Reading of the tests (4h)

Positive reaction: **Red/orange**

Negative reaction: Yellow

Results

1) Differentiation of *Bacteroides thetaiotaomicron* from *B. ovatus*

	ARG
B. thetaiotaomicron	+
B. ovatus	0

2) Differentiation of *Fusobacteria* (see doc. 3.37.0)

	ARG
Fus. mortiferum	+
Fus. varium	+
Fus. necrophorum	0

3) Differentiation of *Prevotella* non-pigmented (see doc. 3.20.2)

4) Differentiation of anaerobe gram-negative rods pigmented (see doc. 3.20.2)

5) Differentiation of the *Bacteroides fragilis* group (see doc. 3.20.2)

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Arginine Aminopeptidase (arginine-β-Naphthylamide)	*B. fragilis* ATCC 25285	*Bacteroides ovatus*

LEUCINE AMINOPEPTIDASE (LAP)

REF No. 46811

The test is based on enzymatic release of beta-naphthylamine from the 1-leucine-beta-naphtahylamid substrate. Beta-naphthylamine is detected by its reaction with Aminopeptidase reagent giving a red colour.

Procedure

Prepare a dense bacterial suspension with a turbidity of at least McFarland No. 4 of the strain to be tested in 0.25 ml saline in a tube. Add one Leucine aminopeptidase tablet and close the tube. Incubate at 35-37 °C for **4 hours**.

After incubation add 3 drops of Aminopeptidase Reagent (92231) and read the colour reaction within 5 minutes. For enterococci/streptococci incubate overnight. See also Aminopeptidase general description in document **3.3.0**.

Reading of the tests (4h)

Positive reaction: **Orange/light orange**

Negative reaction: Yellow

Results

1) Catalase negative gram-positive cocci <u>in clusters</u>

In clusters	LAP	PYR	Van5	ADH	NaCl 6.5%	BE	Remarks
Aerococcus viridans	0	+	S	0	+	60	
Aerococcus urinae	+	0	S	0	+	0	PGUA +
Pediococcus spp.	+	0	R	$+^{0}$	V	+	45°C+
Dolosigranulum pigrum	+	+	S	$+^{0}$	+	0	
Helcococcus kunzii	+	+	S	0	+	0	ONPG+, PGUA+
Gemella (V)	+	$+^{0}$	S	0	0	0	

LAP = Leucine Aminopeptidase Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, Van5 = Vancomycin 5 µg Neo-S (S ≥15 mm, R ≤13 mm), ADH = Arginine Dihydrolase Diatabs™, BE = Bile Esculin Diatabs™, PGUA = Beta-Glucuronidase Diatabs™

2) Catalase negative gram-positive cocci <u>in chains</u>

In chains	LAP	PYR	Van5	ADH	NaCl 6.5%	BE	Remarks
Streptococcus	+	0^{+}	S	V	0	0^{+}	
Enterococcus	+	+	S^{r}	$+^{0}$	+	+	45°C+
Leuconostoc	Owk	0	R	0	$+^{0}$	$+^{0}$	
Abiotropia	+	+	S	V	0	0	αFUC $+^{0}$
Granulicatella	+	+	S	V	0	0	αfuc $+^{0}$
Facklamia	+	V	S	V	+	0	HIP +

Globicatella	0	+	S	0	+	$+^0$	
Gemella (V)	+	$+^0$	S	0	0	0	
Lactococcus	+	69	S	$+^0$	V	$+^0$	45°C 0^+
Vagococcus	$+^0$	+	S	0	V	+	MOT $+^0$

3) *Corynebacteria* non-lipophilic fermentive (most common)

Most strains are: CAT +, MOT 0, Fosfo R, Mupi R, PRO $+^0$, COL R, NALI R.

	Alk P	PZA	αGLU	LAP	AMP	URE	CAMP	NO_3	MAL	O/129	Remarks
C. amycolatum *	+	+	0^+	0	R^s	V	0	$+^0$	80	R	Dry, res
C. argentoratense	V	+	0	82		0	0	0	0	S	
C. coylae	+	+	0	·	S	0	+	0	0	S^r	Clinda R,AlkP
C. diphtheriae	0	0	+	V		0	0	$+^0$	+	S	
*C.glucuronolyticum**	0^+	+	0	+		67	+	V	26	S	PGUA+
C. kutscheri	0	$+^0$	+	+		+	·	$+^0$	+	·	PYR +
C. minutissinum	+	+	0	+	S	0	0	0	+	S	NAG $+^0$
C.pseudotuberculosis	V	0	V	0		+	REV	V	+	R	
C. renale group	0^+	$+^0$	0	0		+	·	0	0	·	PGUA +
C. striatum *	$+^0$	+	0	82	S	0	V	+	0	S	Creamy, res
C. ulcerans	+	0	+	62		+	REV	0	+	V	
C. xerosis	+	+	$+^0$	88	R^s	0	0	60	+	S	dry yellowish
C. hansenii	·	+	0	+	S	0	·	0	+	S	dry yellowish
C. freneyi	+	+	+	+	S	0		V	+	S	wrinkled
C. auriscanis	+	0	0	+	·	0	0	0	0	·	PYR+, HIP+
C. imitans	+	W	0	0		0	+	0	+	R	
C. riegelii					S	$+^R$	0	0	+	S	Glu0, NO3 0

* Resistant or multidrug-resistant

Res= resistance to ≥ 5 drugs

4) Corynebacteria non-lipophilic non-fermentive

Most strains are: CAT +, MOT 0, Fosfo R, Mupi R, PRO $+^0$.

	0/129	LAP	NO_3	CAMP	DNase	Colonies	Remarks
C. afermentans (ANF-1)		0	0	V	0	smooth	
C. auris		+	0	+	0	dry	
Turicella otididis (ANF-1 like)		+	0		+	creamy	
C. propinquum		60	+	0			
C. pseudodiphtericum		$+^0$	+	0			URE +, ERY R
C. coylae	S^r	·	0	+		creamy	URE O, Clinda R, PZA+,Alk P+

PZA = Pyrazinamidase Diatabs™, LAP = Leucine Aminopeptidase Diatabs™, NO_3 = Nitrate Reduction Diatabs™, MAL = Maltose Diatabs™, O/129=O/129 150 µg Diatabs™ (S ≥16 mm, R < 16 mm), NAG = Beta-N- acetylglucosominidase Diatabs™, DNase, URE = Urease Diatabs™, CAT = Catalase, MOT = motility, Fosfo = Fosfomycin Neo-S (R = no zone), Mupi = Mupirocin Neo-S (R = no zone).

5) Globicatella and Aerococcus (3)

	Gram stain	PYR	LAP	Inulin
G. sanguinis	pairs/chains	75	0	93
A. viridans	clusters/tetrads	100	0	7
Enteroc. avium	short chains	95	89	7
Strept. uberis	short chains	100	100	100

PYR = Pyrrolidonyl Aminopeptidase Diatabs™, LAP = Leucine Aminopeptidase Diatabs™, Inulin Diatabs™

6) Aerococci (4) (Vancomycin S, CAT neg. cocci, clusters (dividing in 2 planes)

	PYR	LAP	PGUA	VP	MAL	SUC	ADH	Remarks
Aer. viridans	+	0	V	0^+	V	+	0	
Aer. urinae	0	+	+	0	0	+	0	
Aer. sanguinicola	+	wk+	+	0	+	+	V	
Aer. christensenii	0	+	0	+	0	0	0	
Aer. urinaehominis	0	0	+	0	+	+	0	
Aer. suis (swine)	0	0	0	0	0	0	+	ONPG+,AlkP+

PYR = Pyrrolidonyl Aminopeptidase Diatabs™, LAP = Leucine Aminopeptidase Diatabs™, VP = Voges-Proskauer Diatabs™, MAL = Maltose Diatabs™, SUC = Sucrose Diatabs™

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Leucine Aminopeptidase (L-Leucine-β-naphthylamide-HCL)	*S. bovis* ATCC 15351	*Aerococcus viridans* ATCC 700406

References (LAP)

1) Devriese L.A. et al: Identification of Enterococcus species isolated from foods of animal origin. Intl. J. Food Microbiol. **26**, 187-97, 1995.

2) Renaud F.N.R. et al: Identification of *Turicella otitidis* isolated from a patient with otorrhea associated with surgery: differentation from *Coryneb. afermentans* and *Coryneb. auris*. J. Clin. Microbiol. **34**, 2625-7, 1996.

3) Lynn Shewmaker P. et al: DNA relatedness, phenotypic characteristics and antimicrobial susceptibilities of *Globicatella sanguinis* strains. J. Clin. Microbiol. **39**, 4052-7, 2001.

4) Facklam R. et al: Phenotypic description and antimicrobial susceptibilities of *Aerococcus sanguinicola* isolates from human clinical samples. J. Clin. Microbiol. **41**, 2587-92, 2003.

5) Christensen J.J. et al: Aerococcus urinae: polyphasic characterization of the species. APMIS, **113**, 517-525, 2005.

PROLINE AMINOPEPTIDASE (PRO)

REF No. 46911

The test is based on enzymatic release of beta-naphthylamine from the l-proline-beta-naphthylamide substrate. Beta-naphthylamine is detected by its reaction with Aminopeptidase reagent giving a red colour.

Procedure

Prepare a dense bacterial suspension with a turbidity of at least McFarland No. 4 of the strain to be tested in 0.25 ml saline in a tube. Add one Proline aminopeptidase tablet and close the tube. Incubate at 35-37 °C for **4 hours**.

After incubation add 3 drops of Aminopeptidase Reagent (92231) and read the colour reaction within 5 minutes.

See also Aminopeptidase, general description, in document **3.3.0**

Reading of the tests (4h)

Positive reaction: **Red/orange**

Negative reaction: Yellow

Results

1a) Identification of *Clostridium difficile*

	PRO	***CCFA growth***	***Remarks***
C. difficile	+	+	ONPG 0, PYR 0, Alk P 0, ESC+ Amox S, Merop S, Imipenem I/R
C. inocuum *	0	+	Vanco I/R, Teico S (van B)
C. perfringens	0	·	ONPG +, PYR +, Alk P +
C. ramosum	0	·	
C. sordelli	+	0	
C. bifermentans	+	0	
C. septicum	0	·	

Most clostridia are: Kana 500 S^R, Vanco 5 S, Col R, CAT 0. Metro S.

PRO = Proline Aminopeptidase Diatabs™, CCFA growth = Growth on CCFA medium, ONPG = ONPG Beta- Galactosidase Diatabs™, Alk P = Alkaline Phosphatase Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™,

Amox = Amoxycillin Neo-S, Merop = Meropenem Neo-S, Imipenem = Imipenem Neo-S, Vanco = Vancomycin Neo-S, Teico = Teicoplanin Neo-S.

* *C. inocuum* shows intrinsic low-level resistance to vancomycin (MIC 8-16 µg/ml) with Van 5 zones < 18 mm.

1b) Rapid ID of common lecithinase positive *Clostridium* spp.

	IND	URE	PRO	NAG	Remarks
C. novyi type A	0^{+}	0	0	0	swarm
C. perfringens	0	0	0	+	PYR +, MOT $\underline{0}$
C. bifermentans	+	0	+	V	
C. sordelli	+	+	+	0	

1c) Rapid ID of swarming clostridia

	IND	ESC	PRO	LIP	Remarks
C. novyi type A	0^{+}	0	0	+	Strong beta haem.
C. septicum	0	+	0	0	
C. sporogenes	0	+	+	+	PYR $+^{0}$, NAG 0
C. tetani	$+^{0}$	0	0	0	

URE = Urease Diatabs™, ESC = Esculin Hydrolysis Diatabs™, LIP = Lipase.

1d) Differentiation among Clostridia producing large cytotoxins

	PRO	IND	URE	LEC	Swarm
C. difficile	+	0	0	0	0
C. sordelli	+	+	+	+	0
C. novyi A	0	0^{+}	0	+	+

IND = Indole Diatabs™, URE = Urease Diatabs™, LEC = Lecithinase.

2) Differentiation of Peptostreptococci and similar (most current clinical isolates)

Metro S, Vanco S, Col R

	PRO	PYR	GLU	α-GLU	IND	SPS	Alk P	Remarks
P. anaerobius	+	0	+	+	0	S (≥12 mm)	0	
Peptoniphilus asaccharolyticus,	0	0	0	0	$+^0$	R	+	
Parvimonas micra	$+^0$	+	0	0	0	R	+	
F. magna	0	+	0	0	0	R	V	
P. stomatis	0	0	+	+	0	S (≥15 mm)	0	
Anaerococcus vaginalis	0	0	+	V	0	R	V	LAP+, ADH+
Peptoniphilus harei	0	0	0	0	0	R	0	

PRO = Proline Aminopeptidase Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, α-GLU = Alpha- Glucosidase Diatabs™, IND = Indole Diatabs™, SPS = SPS Diatabs™, GLU = Glucose Diatabs™ (add 3 drops paraffin oil), Alk P = Alkaline Phosphatase Diatabs™

3) Identification of *Candida albicans* (4 hours incubation) (5)

	PRO	NAG	α-GLU (2h)	42 °C
Candida albicans	+	$+^0$	+	+
C. dublinensis	+	$+^0$	0	0
Candida spp. (A)	+	0	-	-
Candida spp. (B)	0	0	-	-

where (A) comprises: *C. guilliermondii*, *C. lipolytica*, *C. lusitaniae*, *C. norvegensis*, *C. parapsilosis*, T*or. candida.*

where (B) comprises: *C. glabrata*, *C. kruseii*, *C. pseudotropicalis*, *C. rugosa* (NAG 0^+), *C. tropicalis* (NAG 0^+).

PRO = Proline Aminopeptidase Diatabs™, NAG = Beta-N-Acetylglucosaminidase Diatabs™, α-GLU(2h) = Alpha- Glucosidase Diatabs™ (2 hours' incubation), 42 °C = Growth at 42 ° in Sabouraud Glucose Agar.

Note: NAG may need overnight incubation to become positive.

4) Test for bacterial vaginosis (7)

Use 0.25 ml vaginal secretion (instead of saline) and add 1 Proline Aminopeptidase Diatabs™.

Incubate for **4 hours** at 35-37°C and add reagent.

	PRO
Gardnerella vaginalis	+
Mobiluncus	+
Atopobium vaginae	+
Candida spp	+[0]
Bifidobacterium	0
Lactobacillus	0

A positive test indicates probable bacterial vaginosis. The presence of Candida spp may give a false positive result.

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Proline Aminopeptidase (L-proline ß-Naphthylamide-HCl)	*P. aeruginosa* ATCC 27853	*Cl. perfringens* ATCC 12917

References (PRO)

1) Garcia A, Garcia T, Pérez J.L.: Proline aminopeptidase test for rapid screening of Clost. difficile. J. Clin. Microbiol. **35,** 3007, 1997.

2) Fedorko D.F. et al: Use of cycloserine-cefoxitin-fructose-agar (CCFA) and l-proline aminopeptidase in the rapid identification of Clostridium difficile. J. Clin. Microbiol. **35**, 1258-9, 1997.

3) Bourgault A.M. et al: Should all stool specimens be routinely tested for Clost. difficile. Clin. Microbiol. Infect. **5**, 219-22, 1999.

4) Murdoch D.A.: Gram-positive anaerobic cocci. Clin. Microbiol. Reviews. **11**, 81-120, 1998.

5) Niimi K. et al: Distinguishing *Candida* species by ß-N-acetylhexosaminidase activity. J. Clin. Microbiol. **39**, 2089-97, 2001.

6) Yuli Song et al: Development of a flow chart for identification of gram-positive anaerobic cocci in the clinical laboratory. J. Clin. Microbiol., **45**, 512-516, 2007.

7) Flores- Paz R. et al: Utility of the system Affirm VP III and the test Proline aminopeptidase for the diagnostic of bacterial vaginosis. Enferm. Infecc. Microbiol. Clin. **26**, 338-342, 2008.

PYRROLIDONYL AMINOPEPTIDASE (PYR)

REF No. 47011

Some bacteria may be differentiated by their ability to enzymatically hydrolize a 1-pyrrolidonyl-beta-naphthylamide substrate. The liberated beta-naphthylamine is identified by reaction with Aminopeptidase reagent producing a red colour in case of positive reactions.

Procedure

Prepare a dense "milky" suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one PYR Diagnostic Tablet, close the tube and incubate at 35-37 °C for 4 hours or up to 18-24 hours. In special cases an incubation period of 1 or 2 hours is used.

After incubation add 3 drops of Aminopeptidase Reagent (92231) and read the colour reaction within 5 minutes. Reading of the test (4h)

Positive reaction: **Red/pink**

Negative reaction: Yellow

Rapid PYR test

A rapid PYR test (1 hour incubation) is performed as follows: colonies of the bacteria to be tested (streptococci, enterococci, staphylococccci, enterobacteriaceae) are suspended in saline (at least McF 4).

Place 1 PYR Diatabs™ in a tube and crush it. Thereafter add 100 microlites (0.1 ml) of the bacterial suspension. Close the tube and incubate for 1 hour at 37°C. Thereafter add one drop of Aminopeptidase reagent and mix. Read the colour development after 1-2 minutes.

Positives

Streptococcus pyogens (group A) Enterococci, Staph. Lugdunensis /S.haemolyticus /S.schleiferi

/S.intermedius Citrobacter/Klebsiella/Enterobacter/Serratia/Yersinia

Results

1) Streptococci (2 hours incubation or 1 hour with the rapid PYR test)

	PYR (2h)
S. pyogenes (haem A)	**+**
Enterococci	**+ (cherry pink colour)**
Other streptococci	**0**

2a) Most common human staphylococci

	PYR (1h)	ODC	Alk P (4h)	POLY	DEFRX
S. aureus	**0**	**0**	**+**	**R (≤12 mm)**	**R (≤14 mm)**
S. epidermidis	**0**	**0⁺**	**+**	**V**	**S (≥16 mm)**
S. haemolyticus	**+**	**0**	**0**	**S (≥14 mm)**	**R**
S. hominis	**0⁺**	**0**	**0**	**S**	**S**
S. lugdunesis	**+**	**+**	**0⁺**	**S**	**R Maltose +**
S. schleiferi	**+⁰**	**0**	**+**	**S**	**R**
S. pseudolugdunensis	**+**	**+**	**V**	**S**	**R Maltose 0**

2b) Coagulase positive staphylococci (9)

	PYR(1h)	ADH	VP (4h)	Poly	MAN (anaer.)	Pigment	Remarks
S. aureus	**0wk**	**V**	**+**	**R (≤12 mm)**	**+**	**+**	
S. intermedius	**+**	**0**	**0wk**	**S (≥14 mm)**	**+**	**0**	
S. pseudintermedius	**+**	**+**	**+⁰**	**S (≥14 mm)**	**0**	**0**	
S. delphini	**+**	**+⁰**	**0**	**S**	**0**	**0**	
S. hyicus	**0**	·	**0**	**V**		**0**	**PGUA+**
S. schleiferi coagulans	**+**	**+**	**+**	**S**		**0**	**MAL 0, SUC 0**

2c) Staphylococci (18-24h)

	PYR (18-24h)
S. aureus	**+**
S. epidermidis	**0**

PYR = Pyrrolidonyl Aminopeptidase Diatabs™, ODC = Ornithine Decarboxylase Diatabs™, VP = Voges-Proskauer Diatabs™, Poly = Polymyxins 150 µg Neo-Sensitabs™ (S ≥ 14 mm, R ≤ 12 mm), Alk P = Alkaline Phospotase Diatabs™, DEFRX = Deferoxamine Diatabs™ (S ≥ 16 mm, R ≤ 14 mm).

3a) Salmonella/Citrobacter (4 hours or 1 hour with the rapid PYR test)

	PYR
Salmonella spp.	**0**
Citrobacter spp.	**+**

3b) Enterobacteriaceae (4 hours, overnight or 1 hour with the rapid PYR test)

	PYR
Citrobacter, Klebsiella, Enterobacter, *Serratia* spp., and most *Yersinia* spp.	**+**
Edwarsiella. *E. coli*, Shigella, Salmonella, *Hafnia* spp., and all the Proteae	**0**

4) Arcanobacterium

	PYR	α−MAN	VP (24h)	TRIB	XYL
A. pyogenes	**82**	**0**	**+**	**0**	**+**
A. haemolyticum	**0**	**+**	**0**	**70**	**0**

5) Vancomycin resistant lactic cocci/coccobacilli from humans

	PYR	BE	ADH	Van5	45 °C
Enterococcus	**+**	**+**	**+⁰**	**S^R**	**+**
Pediococcus	**0**	**+**	**+**	**R**	**+⁰**
Leuconostoc	**0**	**+⁰**	**0**	**R**	**0⁺**
Lactobacillus confusus	**0**	**0**	**+**	**R**	**·**
Lactococcus	**+⁰**	**+**	**+**	**S^R**	**0**

PYR = Pyrrolidonyl Aminopeptidase Diatabs™, α−MAN = Alpha-Mannosidase Diatabs™, VP (24h) = Voges-Proskauer Diatabs™ (incubation 24 h), TRIB = Tributyrin Diatabs™ and XYL = Xylose Diatabs™, BE = Bile Esculin Diatabs™, ADH = Arginine Dihydrolase Diatabs™, Van5 = Vancomycin 5 µg Neo-S (S≥15 mm, R ≤13 mm).

6) Differentiation of H_2S positive (TTR +) members of Enterobacteriaceae

	PYR	LDC	ARA	URE	ONPG
Citrobacter spp.	**+**	**0**	**+**	**V**	**+**
Edwardsiella tarda	**0**	**+**	**0**	**0**	**0**
Leminorella spp.	**·**	**0**	**+**	**0**	**0**
Proteus spp.	**0**	**0**	**0**	**+**	**0**
Salmonella subsp. I	**0**	**+**	**+**	**0**	**0**
Trabulsiella guamensis	**0**	**+**	**+**	**0**	**+**

PYR = Pyrrolidonyl Aminopeptidase Diatabs™, LDC = Lysine Decarboxylase Diatabs™, ARA = Arabinose Diatabs™, URE = Urease Diatabs™, TTR = Tetrathionate Reductase Diatabs™

7) Most common resistant non-fermenters

	TRIB	PYR	TRYP	ACM	TTR	ADH	COL	PSAER	Remarks
Ps. aeruginosa	**+⁰**	**+**	**+**	**+**	**+**	**+**	**S**	**R**	
Ps. fluorescens	**0⁺**	**62**	**+**	**0⁺**	**0⁺**	**+**	**S**	**S**	
Ps. putida	**0⁺**	**0**	**+**	**0**	**0**	**+**	**S**	**S**	
Achr. xylosoxidans	**0**	**+**	**0**	**+⁰**	**+**	**0**	**69**	**S**	
Alc. faecalis	**0**	**0**	**0**	**+**	**+**	**0**	**S**	**S**	**DEFRX S**
Burkh. cepacia complex	**+**	**0**	**0**	**+⁰**	**0**	**0**	**R**	**S**	
Acin. baumanii (OXI 0)	**+**	**0**	**0**	**0**	**0**	**0**	**S^R**	**S**	**NO_3 0, ß-XYL +⁰**
St. maltophilia (OXI 0)	**+**	**0**	**+**	**0**	**+⁰**	**0**	**V**	**S**	**IMIP R, α-MAN +, LDC +**

TRIB = Tributyrin Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, ACM = Acetamide Hydrolysis Diatabs™, TTR = Tetrathionate Reductase Diatabs™, ADH = Arginine Dihydrolase Diatabs™, COL = Colistin 10 µg Diatabs™ (S ≥ 12 mm), PSAER = Ps. aerigunosa Screen Diatabs™ (R ≤ 14 mm), NO3 = Nitrate Reduction Diatabs™, ß-XYL = Beta-Xylosidase Diatabs™, α-Man = Alpha-Mannosidase Diatabs™, IMIP = Imipenem Neo-S, LDC = Lysine Decarboxylase Diatabs™, DEFRX = Deferoxamine Diatabs™ (S ≥ 16 mm, R ≤ 14 mm).

8) Differentiation between *Burkholderia*, *Ralstonia* and *Pandoraea* spp. (Colistin R)

	NO3	OXI	DEF	LDC	PYR	42°C	ONPG	URE	Alk P	Remarks
Burkholderia cepacia complex	**V**	**+**	**RS**	**0**	**+0**	**+0**	**+0**	**V**	**+0**	**ADHO**
Ralstonia spp.	**0⁺**	**+**	**V**	**+**	**0**	**83**	**0**	**+**	**0**	
Pandoraea spp.	**11**	**67**	**·**	**0**	**0**	**89**	**0**	**+**	**+**	**Merop R, Genta R, Tobra R, LAP +, CAT +, MOT +**
Pandoraea sputorum	**0**	**0**	**·**	**0**	**0**	**0**	**0**	**+**	**·**	
Burkholderia gladioli	**33**	**0**	**·**	**0/+**	**0**	**4**	**+**	**+⁰**	**67**	
Burkholderia pseudomallei	**+**	**+**	**S**	**0**	**0**	**0**	**0**	**+⁰**	**+**	**ADH+, GentaR, MOT+**

OXI = Oxidase Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, LDC = Lysine Decarboxylase Diatabs™, URE = Urease Diatabs™, Alk P = Alkaline Phosphatase Diatabs™, Merop = Meropenem Neo-S, Genta = Gentamicin Neo-S, Tobra = Tobramycin Neo-S, LAP = Leucine Aminopeptidase Diatabs™, CAT = catalase, MOT = motility, DEF=Deferoxamine D.T

Quality Control

Diatabs™ (Active ingredients)	Positive	Negative
Pyrrolidonyl Aminopeptidase (L-Pyrrolidonyl-ß-Naphthylamide)	*Enterobacter cloacae* ATCC 13047	*E. coli* ATCC 25922

References (PYR)

1) Wellstood S.A.: Rapid, Cost-Effective Identification of Group A Streptococci and Enterococci by Pyrrolidonyl-beta-Naphthylamide Hydrolysis. J. Clin. Microbiol. 125, 1805-1806, 1987.

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3) Casals J.B., Pringler N.: The value of 3 tests in the identification of staphylococci: pyrrolidonyl aminopeptidase (PYR) and susceptibility towards polymyxins and furazolidone. Staphylococci Symposium. Society Appl. Bacter. Edinburgh, July 1989.

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TRYPSIN (BAA) (TRYP)

REF No. 47211

The test is based on enzymatic release of beta-naphthylamine from the benzoyl-arginin-beta-naphthylamide substrate. Beta-naphthylamine is detected by its reaction with Aminopeptidase reagent giving a red colour. The test is equivalent to the benzile arginine arilmidase (BAA) test.

Procedure

Prepare a dense bacterial suspension with a turbidity of at least McFarland No. 4 of the strain to be tested in 0.25 ml saline in a tube. Add one Trypsin tablet and close the tube. Incubate at 35-37 °C for

4 hours. After incubation add 3 drops of Aminopeptidase Reagent (92231) and read the colour reaction within 5 minutes. See also Aminopeptidase, general description can be found in document

Reading of the tests (4h)

Positive reaction: **Red/orange**

Negative reaction: Yellow

Results

1) *Porphyromonas* (Red fluorescence, Kana R, Vanco S, Oxgall S, CAT 0)

	TRYP	α-FUC	IND
P. asaccharolytica	0^{+}	$+^{0}$	$+^{0}$
P. gingivalis	+	0	+
P. endodentalis	0	0	+
P. catoniae	+	+	0

Downes et al (2) use the following Rosco Diatabs™ in the identification of anaerobic gram-negative bacilli: α-FUC, NAG, β-XYL, α-GLU, TRYP, ESC, ONPG and URE.

2) *Capnocytophaga* spp. (Vanco 5 R, Kana 500 S, Colistin R, Trypsin $+^0$)

	OXI CAT	TRYP	β-XYL	NAG	NO_3	Remarks
C. gingivalis	0	+	0	0	0	RAF $+^0$
C. sputigena	0	+	+	$+^0$	$+^0$	CEL 0
C. haemolytica	0	0 (+)	·	**+**	+	
C. ochracea	0	+	0	+	0	CEL $+^0$
C. granulosa	0	0 (+)	·	0	0	RAF 0^+
C. leadbetteri	0	0	·	**+**	+	SUC 0
C. AHN8471	0	V	·	**+**	0	SUC +
C. cynodegmi (DF-2-like)	+	+	·	**+**	+	ADH +, PYR +, SUC +
C. canimorsus (DF-2)	+	+	·	**+**	0	ADH +, SUC 0

3) IDENTIFICATION OF NON-FERMENTERS, where TRYP (BAA) is a major test (5)

	PYR	TRYP	TRIB	αMAN	LDC	IMP	Remarks
A) OXI 0							
Stenotrophomonas maltophilia	0	+	$+^{0}$	+	+	R	TTR $+^{0}$

	ACM	ALkP	α-GLU	TTR	ADH	DEF(S)	COL(S)	Remarks
B) OXI +, PYR +, TRYP +								
Ps. aeruginosa	+	3	0	+	+	R	100	PSAER (R)
Ps. fluorescens	0^{+}	0	0	0^{+}	+	R	100	PSAER (R)
Sh. putrefaciens	0	100	30	+	0	R	100	
Sh. algae	0	+	.	+	0	R	R	
Elisab. meningoseptica	0	100	+	0	0	R	R	aMAN+, IND$+^{0}$
Sphing. Paucimobilis	0	100	+	0	0	R	19	IND 0, β-XYL+, URE 0, Pigm +
Sphing. Multivorum	0	100	+	0	0	R	R	IND 0, URE +, aMAN+, Bxyl+
O. anthropi	0	0	+	.	36	R	93	URE+, 0/129 S, TOB S
O. intermedium	0	0	+	0	0	.	R	TOB R, URE 0, 0/129 S
O. pseudointermedium	0	0	V	0	0	.	R	TOB R, URE 0, 0/129 S
Inquilinus limosus	.	100	V	.	0	.	R	IND 0, PRO +, NAG +, B-GLU +, NO_3 0, ONPG+, res, mucoid
C) OXI +, PYR +, TRYP 0, NO_3 +								
Ralstonia picketii	0	0	0	0	0^{+}	100	R	MAN 0, NO_3 +
Ralstonia mannitolilytica	0	0	0	0	0	R	R	NO_3 0, MAN +
Com. acidovorans	+	0	0	+	0	R	R	PRO 0, TRIB+
Com. testosteroni	0	0	0	+	0	R	100	PRO 0, TRIB 0
Achr. denitrificans	$+^{0}$	0	0	$+^{0}$	0	R	100	PRO +
Achr. xylosoxidans	$+^{0}$	0	0	$+^{0}$	0	R	69	MOT +, XYL +
Achr. piechaudii	V	0	0	.	0	R	100	PRO 0, NO_3 +, TRIB +
Burkc. gladioli	.	.	.	.	.	R	R	ONPG +, OXI 0, PIGM $+^{0}$
Cupravidus paculus (IVc-2)		+			0	R	S	NO_3 0, URE $+^{R}$

	ACM	ALkP	α-GLU	TTR	ADH	DEF(S)	COL(S)	Remarks
D) OXI +, PYR +, TRYP +								
Ps. fluorescens (PYR 62)	0^{+}	0	0	+	+	R	100	
Sphing. paucimobilis	0	100	+	0^{+}	0	R	19	β-XYL+
Brev. diminuta	0	100	0	+	0	92	R	NO_3 0, yGLU+
Brev. diminuta	0	100	+	+	0	100	R	NO_3 0, yGLU 0
Ps. stutzeri	0 wk	0	$+^{0}$	0	0	R	100	
Ps. alcaligenes	0	0	0	0	+	59	100	NO_3 + PRO 0
Ps. pseudoalcaligenes	0	0	0	0	+	.	100	PRO +
Ps. putida	0	0	0	.	+	R	100	NO_3 0
E) OXI +, PYR 0, TRYP 0,								
*Burkh. cepacia complex *)*	$+^{0}$	87	30	0	0	13	R	LDC $+^{0}$, ONPG $+^{0}$ Bxyl $+^{0}$
Alc. faecalis	+	3	0	$+^{0}$	0	100	100	
Bord. bronchiseptica	0	0	0	0	0	R	100	yGLU+, PRO+, URE+, NO_3+
Olig. ureolytica	0	0	0	V	0^{+}	.	100	yGLU+, URE $+^{R}$, NO_3 +
Olig. urethalis	0	0	0	0	0	100	100	yGLU+, URE 0, NO_3 0
Pandoraea spp.	0	+	0	0	V	.	R	Merop R, LDC 0, ONPG 0, LAP +, CAT +, MOT +

TRYP = Trypsin Diatabs™, α-FUC = Alpha-Fucosidase Diatabs™, β-XYL = Beta-Xylosidase Diatabs™, NAG = Beta-N-Acetyl- glucosaminidase Diatabs™, NO_3 = Nitrate Reduction Diatabs™, IND = Indole Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, TRIB = Tributyrin Diatabs™, α-MAN = Alpha-Mannosidase Diatabs™, LDC = Lysine Decarboxylase Diatabs™, IMP = Imipenem Neo-S (R = no zone), ACM = Acetamide Hydrolysis Diatabs™, Alk P = Alkaline Phosphatase Diatabs™, α- GLU = Alpha-Glucosidase Diatabs™, TTR = Tetrationate Reductase Diatabs™, ADH = Arginine Dihydrolase Diatabs™, DEF = Deferoxamine Diatabs™ (S ≥ 16 mm, R ≤ 14 mm), COL = Colistin 10 µg Diatabs™ (S ≥ 13 mm, R ≤ 10 mm), PSAER = Ps. aeruginosa Screen Diatabs™ (S ≥ 16 mm, R ≤ 14 mm), MAN = Mannitol Diatabs™, NO_3 = Nitrate Reduction Diatabs™, PRO = Proline Aminopeptidase Diatabs™, VP = Voges Proskauer Diatabs™, TTR = Tetrathionate Reductase Diatabs™
, OXI = Oxidase Diatabs™, CAT = catalase, SUC = Sucrose Diatabs™, MOT = motility, URE = Urease Diatabs™, Merop = Meropenem Neo-S, ONPG = ONPG Beta-Galactosidase Diatabs™, LAP = Leucine Aminopeptidase Diatabs™

res = multiresistant.

***) *Burkholderia cepacia* complex (PYR 0, TRYP 0) and similar organisms**

Most strains are GLU +, ADH 0, OXI +wk.

	AlkP	PYR	ESC	NO_3	LDC	ODC	ADH	42°C	ONPG	PIGM	ADON	SUC	Remarks
B. cepacia genom (I)	$+^{0}$	0	V	4	100	30		43	100	82	70	91	
B. multivorans (II)	$+^{0}$	0	V	94	53(V)	0		100	98	2	91	0	
B. cenocepacia (III)	$+^{0}$	0	0	31	99	71		84	99	17	79	90	
B. stabilis (IV)			0	4	100	100		0	0	0	78	0	

B. vietnamensis (V)			0	47	100	0		100	100	0	0	94	
B. dolosa (VI)			0	·	0	0		+	+	·	+	0	
B. ambifaria (VII)			V	V	100	0		26	100	V	+	95	ß haem 84
B. antina (VIII)			0	V	V	0		V	V	0	$+^0$	V	cream colonies
													XYL +, MAL +, LACT +
B. pyrrocinia (IX)			0	$+^0$	+	+	0	V	+	0	+	$+^0$	PRO +, NAG $+^0$
B. ubonensis (X)			0	V	0	0	+	0^+	0	0	0	+	
B.latens			0	0	+	0	0	+	+	0	+	+	
B. diffusa			0	+	+	0	0	$+^0$	+	0	V	+	
B. arboris			V	V	$+^0$	+	0	0^+	+	0^+	+	$+^0$	Bhaem V, XYL0
B. seminalis			(+)	0	$+^0$	$+^0$	0	$+^0$	+	V	+	+	
B. metallica			+	0	+	0	0	+	+	$+^0$	+	+	
Pandoraea spp.	+	0		11	0	0		89	0	0		0	Alk P +, LAP +, CAT +, MOT +, MAL 0, Merop R,
Pandoraea sputorum			R	0	0	0	0	0	0	0	URE+	OXI 0	
B. gladioli	·	+		33	0	0		4	100	77	$+^0$	0	OXI 0, COL R,
B. pseudomallei	+	0	·	+	0	0	+	0	0	0	·	+	DEF S, Genta R
R. picketti	0	+	·	17	0	0		83	0	0	0	0	DEF S, MAL +
Herbaspirillum				·	0				93			0	
B. fungorum	+			+	0	0		0	0	0		0	LAP +, CIT +, TRIB $+^0$
Achr. xylosoxidans	0	+	+	0	0	0	0			0			ACM $+^0$, TTR$+^0$

Note: Most common (> 80 %) in cystic fibrosis patients are *B. multivorans* and *B. cenocepacia* (6).

NO_3 = Nitrate Reduction Diatabs™, LDC = Lysine Decarboxylase Diatabs™, ODC = Ornithine Decarboxylase Diatabs™, 42 °C = growth at 42 °C, PIGM = Pigment production (brown or yellow), SUC = Sucrose Diatabs™, MAL = Maltose Diatabs™, OXI = Oxidase Diatabs™, XYL = Xylose Diatabs™, LACT = Lactose Diatabs™, PRO = Proline Aminopeptidase Diatabs™, NAG = Beta-N-Acetylglucosaminidase Diatabs™, COL = Colistin 10 µg Neo-S (S ≥ 13 mm) DEFRX = Deferoxamine Diatabs™ (S ≥ 16 mm, R ≤ 14 mm). GLU = Glucose Diatabs™, ADH = Arginine Dihydrolase Diatabs™, Alk P = Alkaline Phosphatase Diatabs™, LAP = Leucine Aminopeptidase Diatabs™, CAT = catalase, MOT = motility, Merop = Meropenem Neo-S, CIT = Citrate D.P., TRIB = Tributyrin Diatabs™

4) Differentiation of *Ps. fluorescens*, *Ps. putida*, and *Ps. stuzeri* (Tryp +)

	PYR	α-GLU	TRIB
P. fluorescens	V	0	+

P. putida	0	0	0
P. stutzeri	0	+[0]	+

PYR = Pyrrolidonyl Aminopeptidase Diatabs™, α-GLU = Alpha-Glucosidase Diatabs™, TRIB = Tributyrin

5) Differentiation of *Burkh. cepacia* complex from *B. gladioli*, *Ralstonia pickettii* and *R. manitolilytica*

	PYR	OXI	ONPG	DEF	COL	
Burkh. cepacia complex	0	+ wk	+[0]	R	R	
B. gladioli	+	0	+	R	R	
R. pickettii	+	+	0	S	R	
R. manitolilytica	+	+	0	R	R	MAN +

PYR = Pyrrolidonyl Aminopeptidase Diatabs™, OXI = Oxidase Diatabs™, ONPG = ONPG Diatabs™,

DEF = Deferoxamine Diatabs™, COL = Colistin Diatabs™

6) Differentiation of Chryseobacterium/Elizabethkingia spp.

Most strains PYR+, TRYP+, OXI+, ESC+, IND+ non fermenters

	PIGMred	McConkey	ONPG	URE	IND	ESC
Chryseob. gleum	+	+	0	V	+	+
Chryseob. indologenes	+	V	0	0	+	0
Chryseob. joostei	+	+	0	+	+	
Elizabethk. meningo-septica	0	+	+	V	V	

PIGM= Pigment, McConkey=growth, URE=Urease D. T, IND=Indole D.T, ESC= Esculin hydrolysis

7) Differentiation of most common periodontal pathogens

	TRYP	*aFUC*	*IND*	*NAG*	*Remarks*
Agregibacter actino -mycetecomitans	0	0	0	·	NO_3+ γGLU+, ALA+, LAP+, Clinda R^s
Porphiromonas gingivalis	+	0	+	+	
Prevotella intermedia/nigr.	0	$+^0$	+	0	
Tannerella forsytensis	+	+	V	+	

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Trypsin (Na-Benzoyl-DL-Arginine-ß-Naphthylamide)	*S. maltophilia* ATCC 13637	*E. coli* ATCC 25922

References (TRYP)

1) Summanen P. et. al: Wadsworth Anaerobic Bacteriology Manual. 5th. Ed. Advanced Identification Methods (Level III) pages 49, 50, 65, 93, 158-159 (1993).

2) Downes J. et al: Evaluation of the Rapid ID 32 A system for identification of anaerobic Gram-negative bacilli, excluding the Bacteroides fragilis group. Clin. Microbiol. and Infect. **5**, 319-326, 1999.

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Anaerobes, Presumptive Identification with Oxgall (bile), Brilliant Green and Antibiotic Tablets

A simple screening method is described for separating the major groups of common anaerobic bacteria.

Procedure

Oxgall Diatabs™ (bile) (44311), Brilliant Green Diatabs™ (40511) and the antibiotic tablets: Vancomycin 5µg Neo- Sensitabs (68812), Kanamycin 500 µg Neo-Sensitabs™ (43112), Colistin 10 µg Diatabs™ (66312), and Rifampicin 30 µg Neo-Sensitabs™ (77712) are placed on a plate of FAA + 5% blood or supplemented Brucella Blood Agar, which has been inoculated with an inoculum corresponding to 0.5 McFarland. The plates are incubated anaerobically and the inhibition zones are read after **24-48 hours**.

Results

	NO_3	CAT	Oxgall (bile)	Brilliant Green	Vanco 5 µg	Kana 500 µg	Colistin 10 µg	Rifa Neo-S	Fosfo Neo-S	MOT	Remarks
Bact.fragilis group	0	V	R	S	R	R	R	S	R	0	
Prev.melaninogen./oralis	0	0	S	S	R	R	S^R	S	R	0	
Porphyromonas spp.	0	0	S	S	S	R	R	S	R	0	
Bact.ureolyticus*	+	0	S	S	R	S	S	V	.	0	
Fusob.mortiferum/varium	0	0	R	R	R	S	S	R	S	0	
Other Fusobacteria	0	V	V	R	R	S	S	V	S	0	
Bilophila wadsworthia	+	+	R	.	R	S	S	.	.	+	SIM
Gram positive cocci	V	V	S	.	S	V	R	S	R	.	
Acidominococcus	0	0	S		R	S	S	.	.	0	cocci
Disgonomonas (DF-3)	0	V	R							0	
Ruminococcus	0	0	.		R	R	R			0	
Gram negative cocci	$+^0$	0	S^R	.	R	S	S	S	.	.	
Clostridia spp.	V	0	V	.	S	V	R	S^R	R	$+^0$	
Prevotella massiliensis	0	0	S	.	R	S	.	.	.	0	OXI +
Sutterella wadsworthiensis	+	0	R	.	R	S	S	.	.	0	
Dialister pneumosintes	0	0	S	.	R	S	R	.	.	0	
Synergistes spp.		0	R^S	.	R	S	R	.	.	.	

R = resistant, **S** = sensitive, **S**^{R} = most strains sensitive, **V** = variable, CAT = Catalase, OXI = Oxidase MOT=motility.
**Bact. ureolyticus* is nitrate- and urease-positive.
For Brilliant Green, Kanamycin 500 µg, and Colistin 10 µg: **Sensitive ≥10 mm**; Resistant <10 mm.

For Vancomycin 5 µg: **Sensitive ≥20 mm**; Resistant <18 mm.

For Rifampicin 30 µg Neo-Sensitabs™: **Sensitive ≥16 mm**; Resistant <16 mm.

For Oxgall (bile): **Sensitive: any zone**; Resistant: no zone.

The Oxgall tablets, after incubation, are normally surrounded by a large zone of hemolysis. Organisms growing within this zone of hemolysis (resistant to oxgall) often produce a cloudy precipitate in the agar medium.

Screening of gram-negative anaerobes:

	Vancomycin 5 µg	**Kanamycin 500 µg**	**Colistin 10 µg**	**Fosfomycin**	**Remarks**
Bact. fragilis group	R	R	R	R	OXI 0
Prevotella spp.	R	R	S^{R}	R	OXI 0
Porphyromonas spp.	S	R	R	R	OXI 0
Fusobacterium spp.	R	S	S	S	OXI 0
Prevotella massiliensis	R	S	.	.	OXI +

Quality Control

Diatabs™ (Active ingredients)	Sensitive	Resistant
Oxgall 1000 µg (Oxgall)	*Streptococcus pneumoniae* ATCC 49619	*B. fragilis* ATCC 25285
Brilliant Green 100 µg	*B. fragilis* ATCC 25285	*F. necrophorum* ATCC 25556
Colistin 10 µg (Colistin sulphate)	*E. coli* ATCC 25922	*S. aureus* ATCC 25923

References

1) Draper D.L., Barry A.L.: Rapid identification of Bacteroides fragilis with bile and antibiotic disks. J. Clin. Microbiol. **5**, 439-443, 1977.

2) Leigh D.A., Simmons K.: Identification of non-sporing anaerobic bacteria. J. Clin. Pathol. **30**, 991-992, 1977.

3) Halebian S. et al: Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. J. Clin. Microbiol. **13**, 444-448, 1981.

4) Murray P.R., Citron D.M.: General Processing of Specimens for Anaerobic Bacteria, pp. 488-504 (499-500) in "Manual of Clinical Microbiology" 5th ed., Balows et al (eds.), ASM, 1991.

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6) Anaerobic Gram-negative bacteria, p. 888-896 in Manual of Clinical Microbiology 8th ed. Yolken R.H. et al (eds), ASM 2003.

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Results

	NO_3	**CAT**	**Oxgall (bile)**	**Brilliant Green**	**Vanco 5 µg**	**Kana 500 µg**	**Colistin 10 µg**	**Rifa Neo-S**	**Fosfo Neo-S**	**MOT**	**Remarks**
Bact.fragilis group	0	V	R	S	R	R	R	S	R	0	
Prev.melaninogen./oralis	0	0	S	S	R	R	S^R	S	R	0	
Porphyromonas spp.	0	0	S	S	S	R	R	S	R	0	
Bact.ureolyticus*	+	0	S	S	R	S	S	V	.	0	
Fusob.mortiferum/varium	0	0	R	R	R	S	S	R	S	0	
Other Fusobacteria	0	V	V	R	R	S	S	V	S	0	
Bilophila wadsworthia	+	+	R	.	R	S	S	.	.	+	SIM
Gram positive cocci	V	V	S	.	S	V	R	S	R	.	
Acidominococcus	0	0	S		R	S	S	.	.	0	cocci
Disgonomonas (DF-3)	0	V	R							0	
Ruminococcus	0	0	.		R	R	R			0	
Gram negative cocci	$+^0$	0	S^R	.	R	S	S	S	.	.	
Clostridia spp.	V	0	V	.	S	V	R	S^R	R	$+^0$	
Prevotella massiliensis	0	0	S	.	R	S	.	.	.	0	OXI +
Sutterella wadsworthiensis	+	0	R	.	R	S	S	.	.	0	
Dialister pneumosintes	0	0	S	.	R	S	R	.	.	0	
Synergistes spp.		0	R^S	.	R	S	R	.	.	.	

R = resistant, **S** = sensitive, **S^R** = most strains sensitive, **V** = variable, CAT = Catalase, OXI = Oxidase MOT=motility.
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Porphyromonas spp.	S	R	R	R	OXI 0
Fusobacterium spp.	R	S	S	S	OXI 0
Prevotella massiliensis	R	S	.	.	OXI +

Quality Control

Diatabs™ (Active ingredients)	Sensitive	Resistant
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3) Halebian S. et al: Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. J. Clin. Microbiol. **13**, 444-448, 1981.

4) Murray P.R., Citron D.M.: General Processing of Specimens for Anaerobic Bacteria, pp. 488-504 (499-500) in "Manual of Clinical Microbiology" 5th ed., Balows et al (eds.), ASM, 1991.

5) Bernard D. et al: Bilophila wadsworthia bacteremia in a patient with gangrenous appendicitis. CID, **18**, 1023-4, 1994.

6) Anaerobic Gram-negative bacteria, p. 888-896 in Manual of Clinical Microbiology 8th ed. Yolken R.H. et al (eds), ASM 2003.

ARGININE DIHYDROLASE (ADH)

REF No. 56211

L-arginine is broken down in a two-step process: first from l-arginine to l-citrulline (ADH) followed by a citrulline splitting system. The over-all reaction results in the formation of l-ornithine, CO_2 and NH_3 from the substrate l-arginine, resulting in an alkalinization of the medium and a change of colour of the indicator from yellow to red.

Procedure

Prepare a dense "milky" suspension (at least McFarland No. 4) from the strain to be tested in 0.25 ml saline in a tube. Add one ADH Diagnostic Tablet and **3 drops of sterile paraffin oil**. Close the tube and incubate at 35-37 °C **for 4 hours** or **up to 18-24 hours**.

Reading of the test

Positive reaction: **Red**

Negative reaction: Yellow, yellow orange

After **overnight** incubation, positive reaction: **strong red**; negative reaction: yellow or orange. In most cases overnight incubation is necessary.

Results

1) Enterobacter

Positive: *Enterobacter cloacae*

Usually negative: Other *Enterobacter* spp.

	ADH	MR	Remarks
E. cloacae	97	5	
E. aerogenes	0	5	
E. intermedium	0	100	
E. sakazakii	99	5	α-GLU +
E. agglomerans	0	50	ODC 0

ADH = Arginine Dihydrolase Diatabs™, MR = Methyl Red, α-GLU = Alpha-Glucosidase Diatabs™, ODC = Ornithine Decarboxylase Diatabs™

2) Streptococci/Enterococci

Positive:	*E. faecalis*, *E. faecium*, *E. durans*, *E. gallinarum*, *E. casseliflavus*.
Negative:	Group D streptococci (*Strept. bovis*, *Strept. equinus*) *E. avium*, *E. raffinosus*.

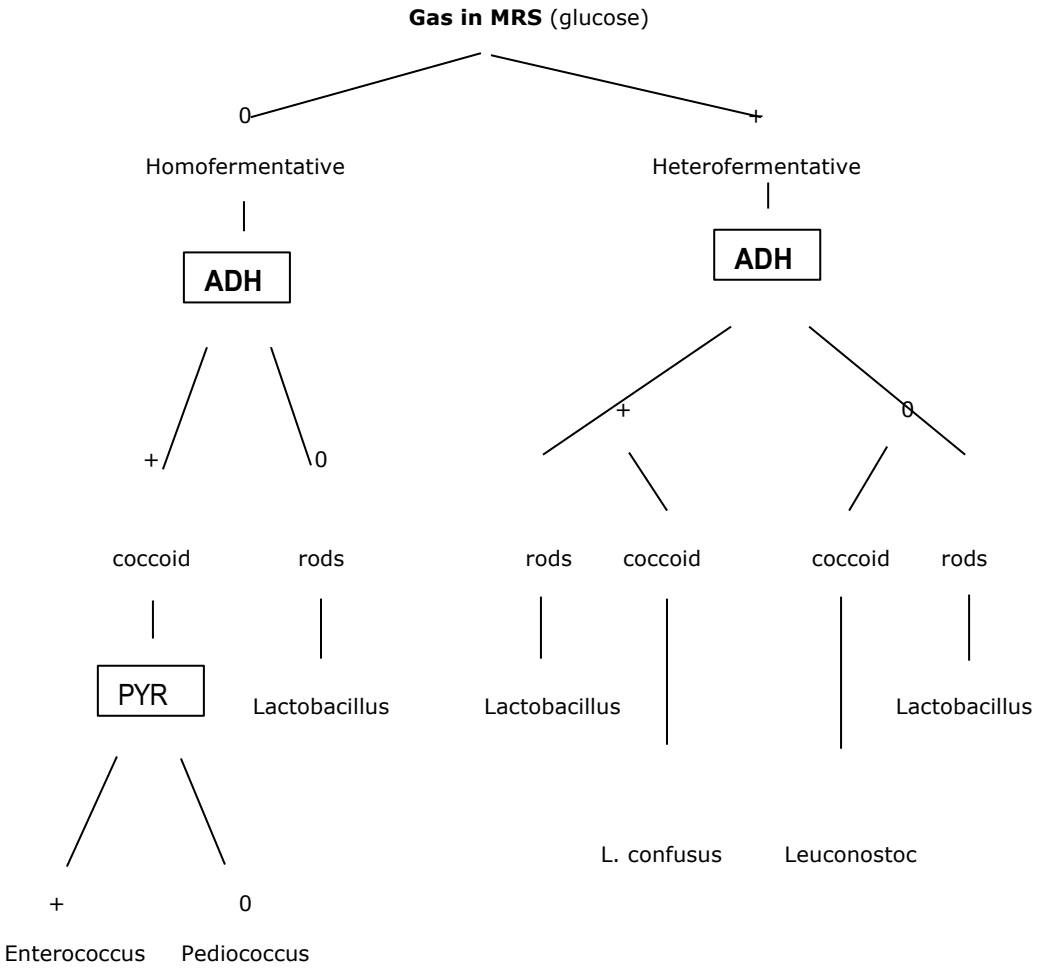
3) Non-fermenters

Positive:	*Ps*. *aeruginosa*, *Ps. fluorescens*, *Ps. putida*, *Ps. pseudoalcaligenes*, *Ps. alcaligenes*, *Ps. stutzeri*, *Cryseom. luteola* (Ve-1).
Negative:	*St. maltophilia*, *Sphing. paucimobilis*, *Shew. putrefaciens*, *Flavobacterium* spp., *Brev. vesicularis*, *Com. acidovorans*, *Com. testosteroni*, *Pasteurella multocida*, *Ralst. pickettii*, *Alcaligenes* spp., *Brev. diminuta*, *Burkh. cepacia*, *Oligella* spp.

4) Staphylococci

Usually positive:	*S. aureus*, *S. haemolyticus*, *S. schleiferi*, *S. simulans*, S. warneri, *S. capitis*.
Usually negative:	*S. hominis*, *S. lugdunensis*, *S. saprophyticus*, *S. xylosus*, *S. cohnii*, *S. sciuri*, *S. lentus.*

5) Identification of lactic bacteria (Vancomycin R)



6) Differentiation of NVS (*Abiotrophia*, *Granulicatella* spp and Helcococcus spp (4)

	ADH	PGUA	NAG	α-GAL	PYR	ONPG
Abiotrophia defectiva	0	0	0	+	+	+
Gran. adjacens	0	+[0]	0	0	+	0
Gran. elegans	+	0	0	0	+	0
Gran. balaenopterae	+	0	+	.	.	.
Helc. kunzii	0	0	.	0	+	+
Helc. sueciencis	0	0	.	0	0	+

NVS = nutritionally variant streptococci, ADH = Arginine Dihydrolase Diatabs™, PGUA = Beta-Glucuronidase Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, MR = methyl red, MRS = Man, Sharp, Rogosa broth, NAG
= N-Acetylglucosaminidase Diatabs™, α-GAL = Alpha-Galactosidase Diatabs™

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Arginine Dihydrolase (L-Arginine HCl)	*P. aeruginosa* ATCC 27853	*K. pneumoniae* ATCC 13883

References

1) Mackey T. et al: Identification of Vancomycin-resistant lactic bacteria isolated from humans. J. Clin. Microbiol. **31**, 2499-2501, 1993.

2) Mohr O'Hara et al: Isolation of Enterobacter intermedium from the gallbladder of a patient with cholecystitis. J. Clin. Microbiol. **36**, 3055-6, 1998.

3) Sato S. et al: Abiotropia elegans comprise 8% of the nutritionally variant streptococci isolated from the human mouth. J. Clin. Microbiol. **37**, 2553-6, 1999.

4) Christensen J.J., Facklam R.R.: Granulicatella and Abiotrophia species from human clinical specimens. J. Clin. Microbiol. **39**, 3520-3, 2001.

BACITRACIN LOW (BaL)

REF No. 40211

Contain a lower amount of bacitracin (0.4 units) than Bacitracin Neo-Sensitabs™ and are specially intended for differentiation of the Lancefield **group A beta haemolytic streptococci** from other **beta-haemolytic streptococci**.

The test is performed on TSA Blood Agar inoculated with the strain to be tested (confluent growth) incubated with 5% CO_2 overnight.

Bacitracin Low Diagnostic Tablets will with group A beta-haemolytic streptococci produce inhibition zones: **≥16mm**, while most beta-haemolytic streptococci from other groups will show smaller or no inhibition zones. Some false sensitive results are seen mainly with streptococci group C and G. Some group C and G streptococci may show zones around 15 mm and can be differentiated from group A streptococci using the Rapid (one hour) PYR test (document 3.3.4, page 1). Only group A are PYR positive.

Results

1) Streptococci

Group A streptococci:	≥16 mm
Other streptococci:	≤15 mm

Bacitracin resistant clones of *S. pyogenes* (group A) were isolated from Belgian and Spanish patients (3,4). Confirm *S. pyogenes* using the PYR test.

Most bacitracin resistant *S. pyogenes* (A) are resistant to erythromycin and clindamycin.

2) *Gardnerella vaginalis*

The test is performed on Mueller-Hinton II agar + 5% blood with an inoculum equivalent to McFarland 0.5

Gardnerella vaginalis:	≥10 mm, PRO +
Bifidobacteria:	< 10 mm
Lactobacilli:	< 10 mm
Streptococci:	< 10 mm

3) Throat cultures

	BaL	PYR	MUPIR	OPT	
Arcanobact. haemolyticum	R	0	R	R	(≤ 16 mm)
Strept. pyogenes A	S^R	+	S	R	
Strept. group C/G	R^S	0	S	R	
Pneumococci	R	0	S	S	(≥ 18 mm)

BaL = Bacitracin low Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, MUPI = Mupirocin Neo-S (S ≥ 16 mm, R < 16 mm), OPT = Optochin Diatabs™

Quality Control

Diatabs™ (Active ingredients)	**Sensitive**	**Resistant**
Bacitracin low 0.4 U	*S. pyogenes* ATCC 12344 *S. pyogenes* ATCC 19615	*S. bovis* ATCC 15351 *S. agalactiae* ATCC 12386

References

1) Stoner R.A.: Bacitracin and coagglutination for grouping of beta-haemolytic streptococci. J. Clin. Microbiol. **7**, 463-466, 1978.

2) Bellon J., Weise B., Verschraegen G., de Meyere M.: Selective Streptococcal Agar Versus Blood Agar for Detection of Group A Beta- Hemolytic Streptococci in Patients with Acute Pharyngitis. J. Clin. Microbiol. **29**, 2084-2085, 1991.

3) Malhotra-Kumar S. et al: Bacitracin-resistant clone of Streptococcus pyogenes isolated from pharyngitis patients in Belgium. J. Clin. Microbiol. **41**, 5282-4, 2003.

4) Montes M. et al: Characterization and evolution of a macrolide and bacitracin-resistant S. pyogenes clone in Spain: 1999-2005. 46th ICAAC, abstract C2-0201, 2006.

BACITRACIN 40 UNITS (BACIT) Neo-Sensitabs™

REF No. 70812

Chocolate blood-agar with a Bacitracin 40 units Neo-Sensitabs™ is useful for the isolation of *Haemophilus* spp. in sputum samples. The test is based on the resistance of *Haemophilus* spp. to high concentrations of bacitracin. Gram positive cocci will show large zones of inhibition around the Bacitracin 40 units tablet, while Haemophilus strains grow near the edge of the tablet (1,2).

Results

BACITRACIN 40 U	**Screening of *Haemophilus* spp. in throat/sputum cultures**
Haemophilus spp.	Growth **very near** the tablet edge
Streptococci/Staphylococci	Growth **far** from the tablet

References

1) Möller L.V.M. et al: N-acetyl-d-glucosamine medium improves recovery of *H. influenzae* from sputa of patients with cystic fibrosis. J. Clin. Microbiol. **31**, 1952-4, 1993.

2) Nye K.S. et al: Incorporated chocolate blood agar and chocolate blood agar plus a bacitracin disk in the isolation of *H. influenzae* from sputum. J. Med. Microbiol. **50**, 472-5, 2001.

BETA LACTAMASE (Acido)

The beta lactamase test (acidometric) is suitable for detecting the production of beta lactamase by the following strains: **Haemophilus**, ***Neisseria gonorrhoeae***, and **staphylococci**.

The test is based on the opening of the beta lactam ring of the substrate (penicillin G) by beta lactamase, resulting in an acidic compound which changes the colour of the indicator (bromcresol purple) from violet to yellow.

Procedure

Prepare a heavy (at least McFarland 4, in some cases 8-10 is needed, unless enzyme induction has been done as per comment below) bacterial suspension in 0.25 ml water or saline in a small tube by picking colonies of the test organism from an overnight plate. A Beta Lactamase Diagnostic Tablet is added. Incubate at 35-37 °C.

Reading of the tests

Positive reaction: The supernatant turns **yellow** (or brownish) within **15-20 min**.*

Negative reaction: Violet

* The reaction time may vary depending upon species, age of culture and the individual strain. A test should not be called negative unless no colour change has taken place in **4 hours**.

Beta Lactamase Induction

It should be noted that some **staphylococci** will not show beta lactamase production, unless the enzyme has been induced by exposure to a beta lactam antimicrobial. In such cases, use growth adjacent to beta lactam antimicrobial tablets (oxacillin, methicillin) or from agar containing beta lactams.

The use of the Beta Lactamase test with strains of Enterobacteriaceae is debatable, because there is lack of correlation between enzyme detection and resistance to beta lactam antibiotics, such as ampicillin, carbenicillin or cephalosporins.

Store at 2-8 °C. Before opening the vial, keep it at room temperature for 1 hour; after opening, store at room temperature (≤25°C) for up to 2 months.

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Beta-Lactamase (Acido) (Penicillinprocaine 4 mg, Penicillin G sodium)	*S. aureus* ATCC 29213	*S. aureus* ATCC 25923

References

1) Shannon K., Phillips I.: Beta-lactamase by 3 simple methods: intralactam, nitrocefin and acidometric, J. Antimicrob. Chemother. **6**, 617-621, 1980.

2) Wegener H.C. et al: Antimicrobial susceptibility of Staph. hyicus isolated from exudative epidermitis in pigs. J. Clin. Microbiol. **32**, 793-5, 1994.

Beta-lactamases (ESBL, AmpC, MBL) detection using Neo-Sensitabs™ and Diatabs™

The detection of different resistance mechanisms in bacteria has in the last years been highlighted by many publications and national recommendations are available in many countries.

Antimicrobial resistance mechanisms including the beta-lactamases are continuously developing and new methods for detection are coming up. Rosco has a broad range of products that in combination may detect different beta-lactamases by phenotypic profiles.

Diatabs™	**Code**	**REF No.**
Cloxacillin 500 µg Diatabs™	CL500	10311
Phenylboronic Acid 250 µg	BORON	10411
Dipicolinic Acid 250 µg	D.P.A	10511

Neo-Sensitabs™ - CLSI potencies		
New cartridges (cartridges with spring)	**Code**	**REF No.**
Aztreonam 30 µg	AZT30	63612
Cefepime 30 µg	FEP30	63712
Cefotaxime 30 µg	CTX30	63912
Cefoxitin 30 µg	CFO30	62912
Cefpodoxime 10 µg	CPD10	63212
Ceftazidime 30 µg	CAZ30	64012
Ceftriaxone 30 µg	CTR30	64212
Amoxycillin+Clavulanate 20+10 µg	AMC30	60112
Cefepime+Clavulanate 30+10 µg	FEP+C	64812
Cefotaxime+Clavulanate 30+10 µg	CTX+C	64712
Cefpodoxime+Clavulanate 10+1 µg	CPD+C	80912
Ceftazidime+Clavulanate 30+10 µg	CAZ+C	64612
Imipenem 10 µg	IMI10	61212
Imipenem+EDTA 10+750 µg	IM+ED	66412
Ticarcillin+Clavulanate 75+10 µg	TIM85	64412
Meropenem 10 µg	MRP10	64312
Ertapenem 10 µg	ETP10	80712

Neo-Sensitabs™		
Cartridges without spring	**Code**	**REF No.**
Aztreonam 30 µg	AZTRM	70712
Cefepime 30 µg	CFEPM	71212
Cefoxitin 60 µg	CFOXT	71712
Ceftazidime 30 µg	CEZDI	72212
Ceftriaxone 30 µg	CETRX	72612
Amoxycillin+Clavulanate 30+10 µg	AM+CL	70212
Cefepime+Clavulanate 30+10 µg	CP+CL	79512
Ceftazidime+Clavulanate 30+10 µg	CZ+CL	72312
Imipenem 15 µg	IMIPM	74612
Imipenem+EDTA 10+750 µg	IM+ED	66412
Ticarcillin+Clavulanate 75+15 µg	TI+CL	78812
Meropenem 10 µg	MEROP	75312
Ertapenem 10 µg	ETP10	80712

Detection of ESBLs using Neo-Sensitabs™

1) **Enterobacteriaceae** (Fig 1)

Strains showing cefotaxime and/or ceftazidime MICs ≥ 2 µg/ml, showing reduced susceptibility to amoxycillin + clavulanate should be tested further for the presence of ESBLs.

Mueller-Hinton agar plates are inoculated with the strain to be tested, and Neo-Sensitabs™ applied onto the agar; Cefotaxime, Ceftazidime and Cefepime Neo-Sensitabs™ at a distance of approx. 20 mm (edge to edge) from Amoxycillin + Clavulanate Neo-Sensitabs™ or using their combinations: Cefotaxime + Clavulanate, Cefepime + Clavulanate and Ceftazidime + Clavulanate Neo-Sensitabs™.

A keyhole or ghost (synergy) zone between Amox + Clav and any of Cefotaxime, Ceftazidime or Cefepime Neo-Sensitabs™ indicates the presence of an ESBL. When using the combination disks, a ≥ 5 mm larger zone for any combination compared to the corresponding single antimicrobial indicates the presence of an ESBL. Always use products from the same range with and without clavulanic acid, e.g. cephalosporins in new cartridges must only be compared to same antimicrobial with clavulanic acid in new cartridges.

Cefpodoxime and Cefpodoxime + Clavulanate may be used for screening purposes.

Klebsiella oxytoca hyperproducing K-1 beta-lactamase may show a false positive result (potentiation of cefotaxime and/or cefepime). Only when the strain is resistant to ceftazidime and shows synergism between ceftazidime and clavulanate should it be reported as ESBL positive.

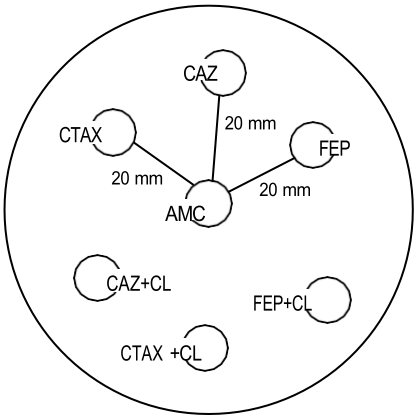


Fig 1. ESBL - Enterobacteriaceae

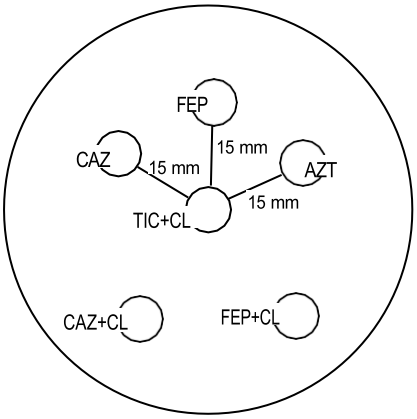


Fig 2. ESBL –

CTAX Cefotaxime, CTAX+CL Cefotaxime+Clavulanic acid, CAZ Ceftazidime, CAZ+CL Ceftazidime+Clavulanic acid, FEP Cefepime, FEP+CL Cefepime+Clavulanic acid, AMC Amoxycillin+Clavulanic acid, AZT Aztreonam, TIC+CL Ticarcillin+ Clavulanic acid.

2) Non-fermenters (Fig 2)

Particularly *P. aeruginosa* and *A. baumannii* may possess several types of beta-lactamases. Non-fermenters showing reduced susceptibility to ceftazidime and/or cefepime and/or aztreonam should be tested for the presence of ESBLs.

Apply Ceftazidime, Cefepime and Aztreonam Neo-Sensitabs™. At a distance of approx. 15 mm (edge to edge) from them apply Ticarcillin + Clavulanate Neo-Sensitabs™. Separately from them apply Ceftazidime + Clavulanate and Cefepime + Clavulanate Neo-Sensitabs™ (double disk synergy test)

A keyhole zone or ghost zone (synergism) between Ticarcillin + Clavulanate and any of Ceftazidime, Cefepime or Aztreonam Neo-Sensitabs™ indicates the presence of an ESBL

With the combination disks a ≥ 5 mm larger zone for Ceftazidime + Clavulanate or Cefepime + Clavulanate compared to the single antimicrobials indicates the presence of an ESBL. Always use products from the same range with and without clavulanic acid, e.g. cephalosporins in new cartridges must only be compared to same antimicrobial with clavulanic acid in new cartridges.

Beceiro et al (12) has shown that the double disk synergy test gives the best results with Acinetobacter spp, due to Acinetobacter's intrinsic susceptibility to clavulanic acid.

Detection of AmpC Beta-lactamases using Neo-Sensitabs™ and Diatabs™

Enterobacteriaceae (Fig 3)

Strains suspicious of possessing plasmid-mediated AmpC beta-lactamases are cefoxitin resistant and have reduced susceptibility to ceftazidime, while currently they are susceptible to cefepime and the carbapenems.

Apply Ceftazidime and Cefoxitin Neo-Sensitabs™. At a distance of 10 mm (edge to edge) from each apply Cloxacillin 500 ug Diatabs™. Apply Ceftazidime + Clavulanate and Cefotaxime + Clavulanate Neo-Sensitabs™. At a distance of 10 mm (edge to edge) apply Boronic Acid Diatabs™.

A keyhole or ghost zone (synergism) between Cloxacillin 500 µg and any of Ceftazidime or Cefoxitin indicates the presence of an AmpC beta-lactamase.

A keyhole or ghost zone (synergism)between Boronic Acid and any of Ceftaxime+Clavulanate or Cefepime + Clavulanate, indicates the presence of an AmpC beta-lactamase.

Inducible AmpC beta-lactamases will show antagonism (distorted zone) between Cefoxitin and Ceftazidime Neo-Sensitabs™. Strains producing plasmid-mediated inducible AmpC enzymes will also show antagonism between cefoxitin and ceftazidime. For further information see leaflet **"Screening and detection of AmpC beta lactamases"**
(www.rosco-diagnostica.com).

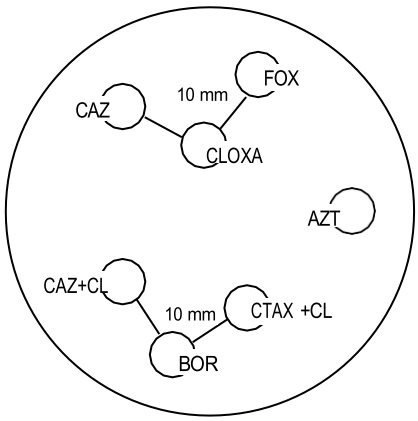


Fig 3 AmpC

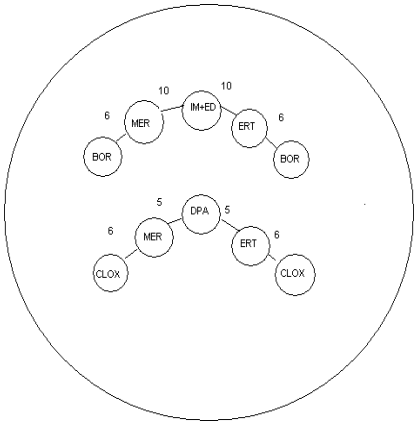


Fig 4 Screening of carbapenemases

(Metallo-β-lactamases and KPC)

FOX Cefoxitin, CLOXA Cloxacillin 500 µg , CAZ Ceftazidime, AZT Aztreonam; CAZ+CL Ceftazidime+Clavulanic acid, CTAX+CL Cefotaxime+Clavulanic acid BOR Boronic acid, AMC Amoxycillin+Clavulanic acid, IMI Imipenem , IMI+EDTA Imipenem+EDTA, MER Meropenem, DPA Dipicolinic acid.

Detection of Carbapenemases using Neo-Sensitabs™ and Diatabs™

See leaflet "Screening and detection of Carbapenemases" (www.rosco-diagnostica.com)

References

1) Vercauteren E. et al: Comparison of screening methods for detection of ESBLs and their prevalence among blood isolates of E. coli and Klebsiella spp. in a Belgian Teaching Hospital. J. Clin. Microbiol. **35**, 2191-2197, 1997.

2) de Gheldre Y. et al: National epidemiologic survey of Enterobacter aerogenes Belgian hospitals from 1996 to 1998. J. Clin. Microbiol. **39**, 889-896, 2001.

3) Tzelepi E. et al: Detection of ESBL in clinical isolates of Enterobacter cloacae and E. aerogenes. J. Clin. Microbiol. **38**, 542-546, 2000.

4) Mirelis B. et al: A simple phenotypic method for the differentiation between acquired and chromosomal AmpC beta-lactamases in E. coli. Enferm. Infecc. Microbiol. Clin. **24**, 370-372, 2006.

5) Wonkeun Song et al: Use of Boronic acid methods to detect the combined expression of plasmid mediated AmpC beta-lactamases and ESBLs in clinical isolates of Klebsiella spp., Salmonella and P. mirabilis. Diagn. Microbiol. Infect. Dis..**57**, 315-318, 2007.

6) Prof P. Nordmann: Evaluation de tests phenotypiques de detection de cephalosporinases integrant l'utilisation des disques de cloxacilline et d'acide boronique. Oct. 2006. Internal study.

7) Ruppe E. et al: First detection of the Ambler Class C1 AmpC beta-lactamase in Citrobacter freundii by a new simple double-disk synergy test. J. Clin. Microbiol. **44,** 4204-4207, 2006.

8) Cornaglia G. et al: Metallo-beta-lactamases as emerging resistance determinants in gram-negative pathogens: open issues. Int. J. Antimicr. Ag. **29**, 380-388, 2007.

9) Franklin C. et al: Phenotypic detection of carbapenem-susceptible metallo-beta-lactamase-producing gram-negative-bacilli. J. Clin. Microbiol. **44**, 3139-3144, 2006.

10) Moland E.S. et al: Prevalence of newer beta-lactamases in gram-negative clinical isolates collected in the U.S. from 2001 to 2002. J. Clin. Microbiol. **44**, 3318-3324, 2006.

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12) Beceiro A. et al: False ESBL detection in Acinetobacter spp. due to intrinsic susceptibility to clavulanic acid. J. Antimicr. Chemother **61**, 301-8, 2008.

13) Bogaerts P. et al: Nosocomial infections caused by multidrug-resistant Ps. Tutida isolates producing VIM-2 Vd VIM-4 metallo-β-lactamases. J. Antimicr. Chemother **61**, 749-751, 2008.

14) Lee K. et al: Effect of Oxgall in the imipenem disk Hodge test in screening MBL producing gram-negative bacilli. ECCMID poster P891, Barcelona 2008.

ESCULIN HYDROLYSIS (ESC) BILE ESCULIN (BE)

REF No. 56611

REF No. 40411

Both tests are based on the demonstration of esculetin released by hydrolysis of esculin. Esculetin reacts with iron to form a brown/black phenolic iron complex. The Bile Esculin Test is mainly used in **differentiating Group D streptococci** and **enterococci** (positive) **from other streptococci** (negative). Esculin Hydrolysis is useful in the differentiation of Streptococci, Enterobacteriaceae, non-fermenters, etc.

Procedure 1

Make a dense suspension of the strain to be tested in 0.25 ml physiological saline with a turbidity of at least McFarland No. 4 in a small tube. Add one Diagnostic Tablet and close the tube. Incubate a 35-37 °C for **4 hours** (or up to **24 hours**). Reading of the tests

Positive reaction:	**Black/grey**
Negative reaction:	Colourless/light grey

Procedure 2

The Diagnostic Tablets are placed onto a blood agar plate inoculated with the strain to be tested. The plate is incubated at 35-37 °C **overnight**.

Reading of the tests

Positive reaction:	The tablet and the colonies around it turn **black/grey** and there is no zone of inhibition (Bile Esculin).
Negative reaction:	The tablet remains white and the colour of the colonies has not changed. A zone of inhibition may appear around the Bile Esculin tablet.

Results

1) *Yersinia enterocolitica* pathogenic serotype

	ESC	SAL	PZA
Yersinia enterocolitica (pathogenic serotype)	0	0	0
Yersinia enterocolitica (non pathogenic)	+	+	+
Yersinia spp.	V	V	+

ESC = Esculin Hydrolysis Diatabs™, SAL = Salicin Diatabs™ and PZA = Pyrazinamidase Diatabs™

All tests performed at **25 °C**.

2) Identification of vancomycin resistant cocci/coccobacilli from humans

	BE	PYR	ADH	Van5	45°C
Enterococcus	+	+	$+^0$	S/R	+
Pediococcus	+	0	$+^0$	R	$+^0$
Leuconostoc	$+^0$	0	0	R	0^+
Lactobac. confusus	0	0	+	R	0
Lactococcus	+	$+^0$	+	S^R	0

BE = Bile Esculin Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, ADH = Arginine Dihydrolase Diatabs™ and Van5 = Vancomycin 5 µg Neo-S (S≥15 mm, R≤12 mm).

3) Differentiation of *S. bovis* I/II, S. gallolyticus, *S. mutans* and *E. faecalis*

	BE	PYR	SORB	MAN	α-GAL	Remarks
S. gallolyticus (S. bovis I)	+	0	0	+	+	URE 0
**S. bovis* II (*S. bovis)*	+	0	0	0	+	URE 0
S. mutans	V	0	+	+	$+^0$	
E. faecalis	+	+	+	$+^0$	0	
S. salivarius group	0	0	·	0	V	URE$+^0$

S. bovis* II comprises: *S. infatarius, S. pasteurianus* (see document **3.36.0)

BE = Bile Esculin Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, SORB = Sorbitol Diatabs™, MAN = Mannitol Diatabs™α-GAL = Alpha-Galactosidase Diatabs™

4) Identification of Actinomyces and related species from human sources
Most strains are: Vanco 5 S, Kana 500 S^R, Col R, Metro R^S, Cipro R.

	PYR	PIGM	CAT	NO_3	CAMP	URE	ESC	αFUC	PGUA	αGLU	NAG	ONPG	ARA	Remarks
A. europaeus		0	0	+	0	0	+	0		+	0	+	0	SUC 0, RAF 0
A. dentalis		0	0	0	·	0	+	0		+	0	+	0	
A. funkei		0	0	+	+	0	0	+		+	+	+	+	
A. georgiae		0	0	V	0	0	+	0		+	0	+	0	SUC +, RAF 0
A. gerencseriae		0	0	V	0	0	+	0		+	0	+	0	SUC +, RAF $+^0$ αMAN +·
A. graevenitzii		+	0	V	0	0	0	0		V	+	+	0	
A. israelii		0	0	+	0	0	+	0		+	0	+	+	αMAN 0
A. meyeri		0	0	V	+	0	0	0		+	+	+	$+^0$	
A. naeslundii		0	0	V	0	+	V	0		+	0	+	0	
A. neuii subsp *neuii*		0	+	+	+	0	0	0		+	0	+	+	
A. neuii subsp *anitratus*		0	+	0	+	0	+	0		+	0	+	0	
A. odontolyticus		+	0	+	0	0	V	V		V	0	+	0	
A. radicidentis		+	+	+	0	0	wk	0		+	0	+	0	
A. radingae		0	0	V	+	0	+	+		+	V	+	+	
A. turicensis		0	0	0	0	0	0^+	V		+	0	0	0	
A urogenitalis		+	0	+	0	0	+	0		+	+	+	wk	
A. viscosus		0	+	+	0	0^+	0	0		+	0	V	0	
Arcanob. bernardiae	V	0	0	0	0	0	0	+	0	+	V	0	wk	
Arcanob. haemolyticum	0^+	0	0	0	$+^{rev}$	0	0	+	0^+	+	+	+	0	
Arcanob. pyogenes	+	0	0	0	0	0	0	0	+	+	0	+	0	
Actinobaculum schaalii	+	0	0	0	wk	0	0	0	0	+	0	0	+	
Actinob. urinale	0	0	0	0	·	+	0	0	+	0	0	0	·	PRO +
Variculum cambriensis		0	0	+	0	0	0	·	·	+	0	V	·	SUC+, RAF0

PIGM = Pigment, CAT = catalase, NO_3 Nitrate reduction Diatabs™, CAMP = CAMP reaction, URE = Urease Diatabs™ ESC = Esculin Hydrolysis Diatabs™, αFUC = Alpha-Fucosidase Diatabs™, αGLU = Alpha-Glucosidase Diatabs™, NAG = Beta-N-Acetylglucosaminidase Diatabs™, ARA = Arabinose Diatabs™, Vanco 5 = Vancomycin 5 µg Neo-S (S ≥ 20 mm, R ≤ 18 mm), Kana 500 = Kanamycin 500 µg Neo-S (S ≥ 10 mm, R < 10 mm), Col = Colistin 10 µg Neo-S (S ≥ 10 mm, R < 10 mm), Metro = Metronidazole 5 µg Diatabs™ (S ≥ 15 mm, R = no zone), PGUA =

Beta-Glucuronisase Diatabs™, PRO = Proline Aminopeptidase Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, Cipro = Ciprofloxacin Neo-S.

5) Differentiation of Leuconostoc and Weisella spp. (Vanco R, PYR 0, LAP 0, BE V)

	ADH	ESC	ARA	RAF
L. mesenteroides subsp. *mesenteroides*	0	+	+	+
L. mesenteroides subsp. *dextranicum*	0	+	0	+
L. citreum	0	+	+	0
L. lactis	0	0	0	+
Weisella paramesenteroides	0	+	+	+
W. confusa	+	+	0	0

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Esculin Hydrolysis (Esculin)	*K. pneumoniae* ATCC 13883	*E. coli* ATCC 25922

References

1) Banton C.E. et al: Abccess caused by vancomycin-resistant Lactobacillus confusus. J.Clin. Microbiol. **29**, 2063-4, 1991.

2) Farmer III J.J. et al: Pyrazinamidase, CR-MOX Agar, Salicin fermentation-Esculin hydrolysis and d-xylose fermentation for identifying pathogenic serotypes of Yersinia enterocolitica. J. Clin. Microbiol. **30**, 2589-94, 1992.

3) Sarkonen N. et al: Phenotypic identification of Actinomyces and related species isolated from human sources. J. Clin. Microbiol. **39**, 3955-61, 2001.

4) Santala A.M. et al: Evaluation of four commercial test systems for identification of Actinomyces and some closely related species. J. Clin. Microbiol. **42**, 418-420, 2004.

C-390

REF No. 41611

An antimicrobial agent, 9-chloro-9-(4-diethylaminophenyl)-10- phenylacridan (C-390) has demonstrated exceptional selective properties for *Pseudomonas aeruginosa* (1,2,3).

C-390 Diagnostic Tablets contain 40 µg diffusible amount per tablet, and are useful for the identification of *Pseudomonas aeruginosa*. C-390 is packed in cartridges of 50 tablets that may be used with a dispenser.

Procedure

Place one C-390 Diagnostic Tablet on an inoculated plate (Mueller-Hinton Agar) for sensitivity testing. Incubate at 35-37 °C for **18-24 hours**. Read the diameter of the inhibition zone in mm. Iso-sensitest Agar may also be used.

Results

	Semi-confluent growth	**Confluent growth (Kirby-Bauer)**
***Pseudomonas aeruginosa*:**	zone <12 mm	no zone
Other *Pseudomonas* spp. and non-fermenters:	zone ≥15 mm	≥12 mm

Some strains of *Alcaligenes xylosoxidans* may give small zones of inhibition with C-390 Diagnostic Tablets.

Quality Control

Diatabs™ (Active ingredients)	**Sensitive**	**Resistant**
C-390 40 µg	*S. maltophilia* ATCC 13637 *E. coli* ATCC 25922 (18-26 mm)	*P. aeruginosa* ATCC 27853 (No zone of inhibition)

References

1) Davis J.R. et al.: "4-h Identification of Pseud. aeruginosa with 9-chloro-9- (4-diethylaminophenyl) -10-phenylacridan". J. Clin. Microbiol. **17**, 1054-1056, 1983.

2) Araj G.F.: "Use of 9-chloro-9-(4-diethylaminophenyl) -10-phenylacridan as a primary medium for recovery of Pseud. aeruginosa from clinical specimens". J. Clin. Microbiol., **20**, 330-333, 1984.

3) Yu P.K.W. et al.: "Comparison of C-390 and cetrimide in the identification of Pseud. aeruginosa". Abstract 624. ICAAC 1985.

4) Casals J.B., Pringler N.: "Identification of Pseudomonas aeruginosa with a C-390 Diagnostic Tablet", 4th European Congress of Clinical Microbiology, Nice, 1989, poster 515.

5) von Graevenitz A. et al.: "Isolation of an unclassified non-fermentative gram-negative rod from a patient on continuous peritoneal dialysis". Eur. J. Clin. Microbiol. Infect. Dis. **12**, 568-570, 1993.

6) Anthony M. et al: Genetic analysis of *Ps. aeruginosa* isolates from the sputa of Australian adult cystic fibrosis patients. J. Clin. Microbiol. **40**, 2772-2778, 2002.

CITRATE (CIT)

REF No. 56511

Diagnostic Tablets for testing alkalinization of citrate. Mainly used in the identification of Enterobacteriaceae and non-fermenting gram-negative bacteria.

Procedure

Prepare a dense bacterial suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one Citrate Diagnostic Tablet and close the tube. Incubate at 35-37 °C for 18-24 hours. Positive reactions can sometimes be observed after 4-6 hours incubation.

Reading of the test

Positive reactions: **Red**

Negative reactions: Yellow/orange

Results

Citrate may be used in the differentiation of Enterobacteriaceae.

CIT positive	
Citrobacter spp.	+
Enterobacter spp.	+
Serratia spp.	+
Providencia spp.	+
Klebsiella pneumoniae/oxytoca	+

CIT negative	
E. coli	0
Shigella spp.	0
Edwardsiella spp.	0
Morganella morganii	0
Proteus vulgaris	0^{+}
Yersinia spp.	0

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Citrate (Citrate)	*P. aeruginosa* ATCC 27853	*Proteus vulgaris* ATCC 13315

References

1) Farmer III J.J. et al: Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. J. Clin. Microbiol. **21**, 46-76, 1985.

CYCLOHEXIMIDE (CYC)

REF No. 58911

Cycloheximide (actidione) is a chemical substance which shows activity against several species of fungi. Cycloheximide Diatabs™ contain 15 µg of diffusible amount per tablet. The difference in sensitivity of **Candida species** to cycloheximide may be useful in the identification of these strains.

Procedure

Place one Cycloheximide Diatabs™on an inoculated plate (Modified Shadomy agar) for sensitivity testing. Incubate at 30-37 °C for **18-24 hours**. Read the diameter of the inhibition zone in mm.

Reading of the tests

Sensitive: **zone ≥25 mm** (MIC ≤16 µg/ml)

Resistant: zone < 25 mm

Results

The following Candida species are **sensitive**: *C.(Tor.) glabrata (S>15 mm)* , *C. krusei*, *C. lusitaniae*. Other sensitive fungi are: *Cryptococcus* spp., *Saccharomyces cerevisiae*.

The following Candida species are found **resistant**: *C. albicans*, *C. pseudotropicalis*, *C. tropicalis*, *C. parapsilosis (V)*, *C. guilliermondii*. Other resistant fungi are: *Trichosporon* spp. and *Geotrichum candidum*. Within the resistant strains, we may differentiate between strains showing a) no zone of inhibition and b) a small zone of inhibition (< 25 mm).

a) No zone: *C. albicans*, *C. pseudotropicalis*

b) Small zone: *C. guilliermondii*, *C. parapsilosis*, *C. tropicalis*

Quality Control

Diatabs™ (Active ingredients)	**Sensitive**	**Resistant**
Cycloheximide 15 µg (Cycloheximide)	*C. krusei* ATCC 6258	*C. albicans* ATCC 64548

References

1) Salkin I.F.: New medium for differentiation of Candida albicans from Candida stellatoidea. J. Clin. Microbiol. **9**, 551-553, 1979.

2) Sobczak H.: A simple disk-diffusion test for differentiation of yeast species. J. Med. Microbiol. **20**, 307-316, 1985.

DEFEROXAMINE (DEFRX)

REF No. 59611

Deferoxamine is a siderophore that has been used in the differentiation of coagulase negative staphylococci.

Deferoxamine Diagnostic Tablets contain 250 µg diffusible amount per tablet and are useful for the identification of *Staphylococcus epidermidis* and *Staphylococcus hominis*.

Principle

Deferoxamine is an iron-chelating agent. Most staphylococci need iron in the media for growth. *S. aureus* grows well under conditions of iron restriction, while most coagulase negative staphylococci need certain amounts of iron in the medium. Deferoxamine Diatabs™ chelates most of the iron around the tablet and consequently particularly *S. epidermidis* and *S. hominis* cannot grow in the vicinity of Deferoxamine Diatabs™, resulting in an inhibition zone, while other staphylococci are not affected.

Procedure: Place one Deferoxamine Diagnostic Tablet on an inoculated plate (Mueller-Hinton II or similar) for sensitivity testing. Incubate at 35-37 °C overnight. Read the diameter of the inhibition zone.

Please note:

1) Use agar media **without** blood. Blood-agar media are useless for this test (iron-chelating).
2) Measure the zone up to colonies of normal size. Particularly with *S. epidermidis* semi-inhibited colonies are found inside the inhibition zone. They should be disregarded.

Results

1) Staphylococci

	DEFRX Zone of inhibition in mm
Staphylococcus epidermidis	≥16 mm (S)
Staphylococcus. hominis	≥16 mm (S)
Staphylococcus lutrae	≥16 mm (S)
Other staphylococci *	≤14 mm (R)

* Other staphylococci include: *S. aureus*, *S. hæmolyticus*, *S. warneri*, *S. simulans*, *S. capitis*, *S. lugdunensis*, *S. schleiferi*, *S. auricularis*, *S. saprophyticus*, *S. xylosus*, *S. cohnii*.

DEFRX = Deferoxamine Diatabs™

2) Coagulase negative staphylococci, human (Powerful discriminating tests)

	DEFRX	Fosfo	Novo	PYR (1h)	ODC
S. epidermidis	S (≥16 mm)	S (≥30 mm)	S (≤14 mm)	0	0
S. hominis	S	R (<28 mm)	S	0	0
S. simulans	R (≤14 mm)	S	S	+	0 , HCF 0
S. haemolyticus	R	R	S	+	0
S. schleiferi	R	S	S	+	0 , HCF +
S. lugdunensis	R	S	S	+	+, Maltose+
S. pseudolugdunensis	R	S	S/R	+	+, Maltose 0
S. saprophlyticus	R	R	R (≤13 mm)	0	0
S. cohnii	R	S	R	0	0
S. xylosus	R	S	R	+	0
S. warneri	R	R	S	0	0
S. capitis	R	R (no zone)	S	0	0

DEFRX = Deferoxamine Diatabs™, Fosfo = Fosfomycin Neo-S, Novo = Novobiocin 5 µg Diatabs™, ODC = Ornithine Decarboxylase D.T, PYR(1h) = Pyrrolidonyl Aminopeptidase Diatabs™ (Incubation 1 hour), HCF =Human Clumping Factor.

3) CNS mastitis staphylococci

	DEFRX	Novo	Fosfo	PYR (1h)	AlkP (4h)
S. hyicus	R (≤14mm)	S (≥14 mm)	S (≥30 mm)	0	+
S. chromogenes	R	S	S	V	+
S. simulans	R	S	S	+	V
S. warneri	R	S	R	V	0
S. haemolyticus	R	S	R (<28mm)	+	0
S. epidermidis	S (≥16 mm)	S	S	0	+
S. hominis	S	S	R	0	0
S. xylosus	R	R (<13mm)	V	+	V

DEFRX = Deferoxamine Diatabs™, Novo = Novobiocin 5 µg Diatabs™, Fosfo = Fosfomycin Neo-S.

4) Coagulase positive staphylococci

	DEFRX	Poly	VP(4h)	MAL	TRE	PYR (1h)
S. aureus	R (≤14mm)	R (≤12mm)	+	+	+	0 wk
S. intermedius	R	S (≥14 mm)	0	0w	+	+
S. pseudintermedius	R	S (≥14 mm)	+	+	+	+
S. schleiferi (coagulans)	R	S	+	0	0	+
S. hyicus	R	S	0	0	+	0
S. delphini	R	S	0	+	0	+
S. lutrae	S (≥16 mm)	S	0	+	+	·

DEFRX = Deferoxamine Diatabs™, Poly = Polymyxin Neo-S, VP(4h) = Voger Proskauer Diatabs™ (4 hours incubation), MAL = Maltose Diatabs™, TRE = Trehalose Diatabs™, PYR (1h) = Pyrrolidonyl Aminopeptidase Diatabs™ (1 h incubation).

5) Cupriavidus (7,8) (Wautersia) Most strains are: CAT +, Oxi +, PYR +, TRYP 0.

	COL10	DEFRX	MAN	Alk P	URE
Ralstonia pickettii	R	S	0	0	$+^{0}$
R. mannitolilytica	R	S	+	0	+
Cupriavidus insidiosus	R	R	0	0	wk
Cupriavidus gilardii	S	R	0	+	0
Cupriavidus pauculus (IVc-2)	S	R	0	+	$+^{R}$

COL10 = Colistin 10 µg (S ≥13 mm, R ≤ 10 mm), DEFRX = Deferoxamine Diatabs™, (S ≥16 mm), MAN = Mannitol Diatabs™, Alk P = Alkaline Phosphatase Diatabs™, URE = Urease Diatabs™, $+^{R}$ = rapid positive, CAT
= catalase, OXI = Oxidase, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, TRYP = Trypsin Diatabs™

6) Screening tests for *Burkholderia pseudomallei* (9)

The following results are presumptive of *B. pseudomallei*:

- Gram negative rods with bipolar staining
- Metallic sheen
- Oxidase+
- Colistin R, Gentamicin R
- Trypsin neg, PYR neg.
- DEFEROXAMINE S

It can be differentiated from *B. cepacia* complex, as *B. cepacia* shows Deferoxamine R and are ADH neg, while *B. pseudomallei* are DEF S and ADH+.

Quality Control

Diatabs™ (Active ingredients)	**Sensitive**	**Resistant**
Deferoxamine 250 µg (Deferoxamine mesylate)	*S. epidermidis* ATCC 12228	*S. aureus* ATCC 25923

References

1) Lindsay J.A., Riley T.V.: Susceptibility to desferrioxamine: a new test for the identification of Staphylococcus epidermidis. J. Med. Microbiol., **35**, 45-48, 1991.

2) Devriese L.A. et al: A simple identification scheme for coagulase negative staphylococci from bovine mastitis". Research in Vet. Science **57**, 240-4, 1994.

3) Mulder J.G.: A simple and inexpensive method for the identification of Staph. epidermidis and Staph. hominis. Eur. J. Clin. Microbiol. Infect. Dis. **14,** 1052-6, 1995.

4) Foster G. et al: Staph. lutrae sp. nov. of new coagulase-positive species isolated from otters. Intl. J. Syst. Bacteriol. **47**, 724-6,1997.

5) Kahlmeter G. et al: S.lugdunensis - orsakar inte bara endokardit, 1998.

6) Nuttall N.: Identification of clinically significant coagulase negative staphylococci. Workshop 4th South Pacific Congress 9-13 October 1995.

7) De Baere T. et al: Classification of Ralstonia pickettii biovar 31 "thomasii" strains and of new isolates related to nosocomial recurrent meningitis as Ralstonia mannitolilytica sp. nov. IJSEM **51**, 547-558, 2001.

8) Vay C. et al: Bacteremia due to Cupriavidus pauculus (formerly CDC group IVc-2) in a haemodyalisis patient. Clin. Microbiol. Newsletter, **29**, 30-32, 2007.¨

9) Laffineur K. et al: Biochemical- and susceptibility tests useful for identification of nonfermanting gram-negative rods. J. Clin. Microbiol. 40, 1085-7, 2002.

DOUBLE TEST Diatabs™

Double Test Diatabs™ permit performing **two tests** using **one tablet**.

Double test reactions are read as follows:

After incubation for **4 hours** or (**18-24 hours**) at 35-37°C

a) the first reaction is read **without reagent** addition providing the first test result, and

b) in the same tube the second reaction is read **after reagent addition**, providing the second test result.

The following Double Test Diatabs™ are currently available:

Lysine decarboxylase (LDC)/Indole	Enterobacteriaceae
ODC/Indole	Enterobacteriaceae
PGUA/Indole	*E. coli*
Urease/Indole	Enterobacteriaceae, Non-Fermenters
Urease/TDA	Enterobacteriaceae

The use of simplified rapid testing results in up to 75 % reduction in cost of reagents and technologist time, with a decrease in time to reporting.

LDC / INDOLE (LDC/IND)

REF No. 58411

Double Test tablet for Lysine decarboxylase (LDC) and Indole test, mainly for use in the identification of **Enterobacteriaceae**.

Procedure

Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a small tube. Add one diagnostic tablet and **3 drops of paraffin oil** and close the tube. The oil overlayer provides anaerobic conditions necessary to avoid false positive reactions for the lysine decarboxylase test. Incubate at 35-37 °C for **3-4 hours** (or **up to 24 hours**).

Reading of the tests

Lysine decarboxylase (LDC)

NB! The Lysine decarboxylase test must be read before adding reagent for the Indole test.

Positive reaction:	**Blue/violet**
Negative reaction:	Yellow, green, grey

After **overnight** incubation **only strong blue or violet** is positive!

Indole

After reading the LDC test add **3 drops of Kovacs' reagent** (92031), shake gently and wait for 3 minutes. Look only at the **colour of the surface layer**.

Positive reaction:	**Red** (surface layer)
Negative reaction:	Yellow

Results

1) Screening for Salmonella/Shigella (1). LOUIS Test (3 hours)

LDC	ONPG	URE	IND	Possible ID	Step 1	Step 2
+	+	0	+	*E. coli*	Discard	
+	0	0	+			
0	0	+	+	*Proteus* spp.	Discard	
0	0	+	0	Morganella		
+	0	0	0	Salmonella	Confirm by serology	Neg. Discard
0	0	0	0	*Shigella* spp. (LDC neg. Salmonella)	Confirm by serology	Neg. Discard
0	0	0	+	*Shigella* spp.	Confirm by serology	Neg. Discard
0	+	0	0	*Shigella sonnei* or *Sh. dysent. I*	Confirm by serology	Neg. Discard

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
LDC/Indole (L-Lysine, L-Tryptophane)	*E. coli* ATCC 25922 (LDC pos., IND pos.)	*Proteus vulgaris* ATCC 13315 (LDC neg., IND pos.) *K. pneumoniae* ATCC 13883 (LDC pos., IND neg.)

References

1) Wilson G.: Rapid and economical method for biochemical screening of stool isolates for Salmonella and Shigella species. J.Clin. Microbiol. **42**, 4821-3, 2004.

ODC / INDOLE (ODC/IND)

REF No. Non-stock
(59111)

Double Test tablet for Ornithine decarboxylase (ODC) and Indole test, mainly for use in the identification of Enterobacteriaceae.

Procedure

Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a small tube. Add one diagnostic tablet and **3 drops of paraffin oil** and close the tube. The oil overlayer provides anaerobic conditions necessary to avoid false positive reactions for the ornithine decarboxylase test. Incubate at 35-37 °C for **3-4 hours** (or **up to 24 hours**).

Reading of the tests

Ornithine decarboxylase (ODC)

NB! The Ornithine decarboxylase test must be read before adding reagent for the Indole test.

Positive reaction: **Blue/violet**

Negative reaction: Yellow, green, grey

After **overnight** incubation **only strong blue or violet** is positive!

Indole

After reading the ODC test add **3 drops of Kovacs' reagent** (92031), shake gently and wait for 3 minutes or more. Look only at the **colour of the surface layer**.

Positive reaction: **Red** (surface layer)

Negative reaction: Yellow

Results

1) Differentiation of *Citrobacter* spp.

	ODC	IND	DUL	ESC	MALON	MEL	RAF	Remarks
C. freundii	0	0	0^+	0	0	+	$+^0$	
C. koseri	+	+	V	0^+	+	0	0	ADON $+^0$
C. amalonaticus	+	+	0	0^+	0	0	0	ß-XYL V

C. braaki	+	0	V	0	0	+	0
C. farmeri	+	+	0	0+	0	+	+
C. gillenii	0	0	0	V	+	+0	0+
C. murliniae	0	+	+	V	0	V	0+
C. sedlakii	+	+	+	+	+	+	0
C. werkmanii	0	0	0	0	V	0	0
C. youngae	0+	0	+0	0	0+	0	0

ODC/IND = ODC/Indole Diatabs™, DUL = Dulcitol Diatabs™, ESC = Esculin Hydrolysis Diatabs™, MALON = Malonate, MEL = Melibiose Diatabs™, RAF = Raffinose Diatabs™, ADON = Adonitol Diatabs™, -XYL = Beta-Xylosidase Diatabs™

2a) Differentiation of biotypes of *H. influenzae* (4)

	ODC	IND	URE
Biotype I	+	+	+
Biotype II	0	+	+
Biotype III	0	0	+
Biotype IV (*H. quentini)*	+	0	+
Biotype V	+	+	0
Biotype VI	+	0	0
Biotype VII	0	+	0
Biotype VIII	0	0	0

ODC/IND = ODC/Indole Diatabs™, URE = Urease Diatabs™

2b) Differentiation of H. influenzae and H. haemolyticus

	ODC	Bhaem.
H. influenzae	+0	0
H. haemolyticus	0	+0

3) Differentiation of most common *Vibrio* spp. (human interest)

Most *Vibrio* spp. are OXI +, O/129 S, NO_3 +. Inoculum on 2.5 % NaCl solution, incubation at 30 °C.

	γGLU	IND	ADH	LDC	ODC	ONPG	ARA	MAN	PRO	VP	COL	Remarks
Vibrio cholerae classical	·	+	0	+	+	+	0	+	0^{+}	0	S	TTR 0
Vibrio cholerae El Tor	·	+	0	+	+	+	0	+	0^{+}	+	R	
Vibrio mimicus	+	+	0	+	+	$+^{0}$	0	+	0	0	S^{R}	
Vibrio metschnikovii	·	20	60	35	0	50	0	+	·	+	S	OXI 0, NO_3 0
Grimontea (V) hollisae	·	+	0	0	0	0	+	0	0	0	S	NAG 0^{+}, PYR +
Photobacterium (Vibrio) damsela	0	0	$+^{0}$	50	0	0	0	0	0	$+^{0}$	S^{R}	PYR +
Vibrio fluvialis/ V. furnisii	+	0^{+}	$+^{0}$	0	0	40	$+^{0}$	+	+	0	S	
Vibrio alginolyticus	+	$+^{0}$	0	+	50	0	0	+	+	+	R^{S}	TRYP +, TTR +
Vibrio parahaemolyticus	+	+	0	+	+	0^{+}	80	+	+	0	R^{S}	TRYP +, TTR +
V. vulnificus bio 1	0	+	0	+	$+^{0}$	V	0	$+^{0}$	+	0	R	SORB 0
V. vulnificus bio 2	0	V	0	+	0	V	0	0	·	0	R	SORB +
V. vulnificus bio 3	0	+	0	+	+	V	0	0	·	0	R	SORB 0
Vibrio harveyi	+	+	0	+	0	0	0	50	·	50	R	

ADH = Arginine Dihydrolase Diatabs™, LDC = Lysine Decarboxylase Diatabs™, ARA = Arabinose Diatabs™ , MAN = Mannitol Diatabs™, PRO = Proline Aminopeptidase Diatabs™, VP = Voges Proskauer Diatabs™, COL = Colistin 10 µg Neo-S (S ≥ 13 mm, R = ≤ 10 mm), TTR Tetrathionate Reductase Diatabs™, NO_3 = Nitrate Reduction Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, TRYP = Trypsin Diatabs™, O/129 Diatabs™ (S ≥ 16 mm, R < 16 mm), γGLU=Gamma Glutamyl Aminopeptidase Diatabs™

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
ODC/Indole **(L-Ornithine, L-Tryptophane)**	*E. coli* ATCC 25922 (ODC. pos, IND pos.)	*K. pneumoniae* ATCC 13883 (ODC neg., IND neg.)

References

1) Brenner D.J. et al: Classification of Citrobacteria by DNA hybridization: Designation of C. farmeri sp. nov., C. youngae sp. nov., C. braakii sp. nov., C. werkmanii sp. nov., C. sedlakii sp. nov. and 3 unnamed Citrobacter genomospecies. Intl. J. Syst. Bacteriol. **43**, 645-658, 1993.

2) Janda M.J. et al: Biochemical identification of Citrobacteria in the clinical laboratory. J. Clin. Microbiol. **32**, 1850-4, 1994.

3) Brenner D.J. et al: Biochemical identification of Citrobacter species defined by DNA hybridization and description of Citrobacter gillenii sp. nov. and C. murliniae sp. nov.. J. Clin. Microbiol. **37**, 2619-24, 1999.

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6) Mak G.C. et al: Reduced levofloxacin susceptibility and tetracycline resistance in a clinical isolate of Haemophilus quentini identified by 16S or RNA sequencing. J. Clin. Microbiol. **43**, 5391-5392, 2005.

PGUA / INDOLE (PGUA/IND)

REF No. 59011

Double Test tablet for Beta Glucuronidase (PGUA) and Indole test, mainly for use in the identification of ***Escherichia coli*** e.g. from urinary tract infections.

Approx. 94 % of *E. coli* are positive for PGUA and approx. 99 % are positive for Indole.

The use of simplified identification systems saves laboratory resources, results in up to 75% reduction in cost of reagents and technologist time with a reduction in time to reporting (4).

Procedure

Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a small tube. Add one diagnostic tablet and close the tube. Incubate at 35-37 °C for **3-4 hours** (or **up to 24 hours**).

Reading of the tests

Beta Glucuronidase (PGUA)

NB! The Beta Glucuronidase test must be read before adding reagent for the Indole test.

Positive reaction:	**Yellow**
Negative reaction:	Colourless

Indole

After reading the PGUA test add **3 drops of Kovacs' reagent** (92031), shake gently and wait for 3 minutes. Look only at the **colour of the surface layer**.

Positive reaction:	**Red** (surface layer)
Negative reaction:	Yellow

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
PGUA/Indole (p-Nitrophenyl-β-D-Glucuronic acid, L-Tryptophane)	*E. coli* ATCC 25922 (PGUA pos., IND pos.) *Proteus vulgaris* ATCC 13315 (PGUA neg., IND pos.)	*Enterobacter cloacae* ATCC 13047 (PGUA neg., IND neg.)

References

1) Iritani B. et al: Evaluation of a rapid tube assay for presumptive identification of E. coli from veterinary specimens". J. Clin. Microbiol. **26**, 564-6, 1988.

2) Casals J.B., Pringler N.: Rapid Identification of E. coli with a Double Test Tablet: Beta Glucuronidase (PGUA)/Indole. 4th European Congress of Clinical Microbiology, Nice, 1989, poster 514.

3) Domínguez A., Alcaide F., Pulido A., Ayats J., Pérez J.L., Martín R.: Use of a Commercial Double-Test Tablet (Rosco PGUA/Indole) for Screening of Escherichia coli. Diagn. Microbiol. Infect. Dis. **15**, 291-294, 1992.

4) York M.K. et al.: Multilaboratory validation of rapid spot tests for identification of E.coli. J. Clin. Microbiol. **38**, 3394-8, 2000.

UREASE / INDOLE (URE/IND)

REF No. 57611

Double Test tablet for the Urease test and the Indole test; both tests are commonly used in identification of e.g. **Enterobacteriaceae** and **non- fermenting gram-negative bacteria**.

Procedure

Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one diagnostic tablet and close the tube. Eventually, add. 3 drops of paraffin oil and incubate at 35-37 °C for **4 hours** (or **18-24 hours**). For "non-fermenters" overnight incubation is recommended.

Reading of tests

Urease

NB! The urease test must be read before adding reagent for the Indole test.

Positive reaction:	**Red/purple**
Negative reaction:	Yellow

After overnight incubation only strong red/purple is positive!

Indole

After reading the Urease test add **3 drops of Kovacs' reagent** (92031), shake gently and wait for 3 minutes. Look only at the **colour of the surface layer**.

Positive reaction:	**Red** (surface layer)
Negative reaction:	Yellow/orange

Results

1) Differentiation of *Actinobacillus* spp. from *Pasteurella* spp./*Mannheimia* spp. (CAT +, OXI+)

	URE	IND	α-GLU	SUC	Remarks
Actinobacillus spp.	+	0	V	+	
Pasteurella spp.	0^+	+	+	+	
Mannheimia spp.	0	0	0	+	
Haemophilus spp.	V	V	0	0	Factor X/V +

α-GLU = Alpha-Glucosidase Diatabs™ SUC = Sucrose Diatabs™, Factor X Diatabs™, Factor V Diatabs™

2) Differentiation of *Pasteurella* spp. (human interest)

Most strains are: OXI +, CAT +, NO_3 +, ADH 0, URE 0, ESC 0, O/129 S, MOT 0.

Colonies typical sweetish smell of indole, non-haemolytic.

	CAT	IND	URE	ODC	ONPG	MAL	TRE	MAN	SOR	Remarks
"P. caballi"	0	0	0	+	+	$+^0$	0	+	0	
P. canis bio 1	+	+	0	+	0	0	$+^0$	0	0	
P. canis bio 2	+	0	0	+	0	0	+	0	0	
P. dagmatis	+	+	+	0	0	+	+	0	0	
P. langaaensis	0	0	0	0	+	0	0	+	0	
P. multocida ssp. *multocida*	+	$+^0$	0	$+^0$	0	0^+	$+^0$	$+^0$	+	DUL 0, α-GLU +
P. multocida ssp. *septica*	+	+		0	0	0^+	+	+		DUL 0, α-GLU +
P. multocida ssp. *gallicida*	+	+		0	0	0^+	0	+		DUL +, α-GLU 0
Taxon 45 Bisgaard	+	+		0	0	0^+	0	0		α-GLU 0^+, SUC 0
P. stomatis	+	$+^0$		0	0	0	+	0		
Gallibacterium anatis	+	0		0	+	V	$+^0$	+		α-GLU +
Avibacterium avium	$+^0$	0		0	0	0	+	0		
Avibacterium gallinarum	+	0		0	0	+	+	0		
Avibacterium paragallinarum	0	0		0	0	V	0	+		

CAT = catalase, URE/IND = Urease/Indole Diatabs™, ODC = Ornithine Decarboxylase Diatabs™, MAL = Maltose Diatabs™, TRE = Trehalose Diatabs™, MAN = Mannitol Diatabs™, SOR = Sorbitol Diatabs™, DUL = Dulcitol Diatabs™, α-GLU = Alpha-Glucosidase.

3) Differentiation of *clostridia* producing neurotoxins (Gel +)

	IND	LEC	ESC	NO_3	URE
C. tetani	75	0	0	0	0
C. botulinum type B	0	0	+	0	+
Clostridium spp. RKD	+	+	0	+	+

Gel = gelatinase, LEC = lecithinase, ESC = Esculin Hydrolysis Diatabs™, NO_3 = Nitrate Reduction Diatabs™, URE/IND = Urease/Indole Diatabs™

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Urease/Indole (Urea, L-Tryptophane)	*Morganella morganii* ATCC 25830 (URE pos., IND pos.) *K. pneumoniae* ATCC 13883 (URE pos., IND neg.)	*E. coli* ATCC 25922 (URE neg., IND pos.)

References

1) Ashhurst-Smith C. et al.: Actinobacillus equuli septicemia: an unusual zoonotic infection. J. Clin. Microbiol. **36**, 2789-90, 1998.

2) Euzéby J.P. Dictionnaire de bacteriologie veterinaire. March 2001.

3) Gerards S.H. et al: Pasteurella multocida ssp. multocida and P. maltocida ssp. septica. Differentiation by PCR fingerprinting and α-glucosidase activity. J. Clin. Microbiol. **39**, 2558-64, 2001.

4) Aparma Dixit et al: Characterization of Clostridium spp. RKD producing botulinum–like neurotoxin. System. Appl. Microbiol. **28**, 405-414, 2005.

UREASE / TDA (URE/TDA)

REF No. 57911

Double Test tablet for the Urease test and the Tryptophane deaminase test (TDA). The tablet is mainly used in identification of **Enterobacteriaceae** and is especially useful in differentiation of the

Proteus-Morganella-Providencia-group (TDA positive) from the rest of the family.

Procedure

Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a small tube. Add one diagnostic tablet and close the tube. Incubate at 35-37 °C for **3-4 hours** (or **18-24 hours**).

Reading of the tests

Urease

NB! The Urease test must be read before adding reagent for the Tryptophane deaminase test.

Positive reaction:	**Red/purple**
Negative reaction:	Yellow

After overnight incubation only strong red/purple is positive!

Tryptophane deaminase (TDA)

After reading the Urease test add **2 drops of Ferric Chloride 10% solution** and read within 5 minutes.

Positive reaction:	**Red/brown**
Negative reaction:	Yellow/orange

Indole-positive strains may produce an orange colour due to indole production. This is a negative reaction.

Results

	URE	TDA
Proteus spp.	$+^R$	+
Morganella spp.	$+^R$	+
Providencia spp.	V	+
Other Enterobacteriaceae	V	0

$+^R$ = rapid positive reaction

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Urease/TDA (Urea, L-Tryptophane)	*Proteus vulgaris* ATCC 13315 (URE pos., TDA pos.) *K. pneumoniae* ATCC 13883 (URE pos., TDA neg.)	*E. coli* ATCC 25922 (URE neg., TDA neg.)

GENTAMICIN 250 µg (GN250), KANAMYCIN 500 µg (KA500), STREPTOMYCIN 500 µg (ST500) Neo-Sensitabs™

REF No. 43012

REF No. 43112

REF No. 44712

High content tablets for detection of **high-level resistance (HLR) towards the aminoglycosides** in enterococci and streptococci.

Kanamycin 500 µg is also useful in the presumptive identification of anaerobes.

In several countries approx. 50 % of *E. faecalis* isolates are highly resistant to streptomycin (MIC >2000 µg/ml) and HLR to gentamicin is increasing rapidly. Low content discs and automatized methods have difficulties in detecting this kind of resistance.

Procedure

The media recommended are: Mueller-Hinton II **without blood for enterococci** and M-H II with 5% blood for streptococci. The inoculum is standardized as for routine sensitivity testing (0.5 McFarland).

Reading of the tests

Zone diameters and the corresponding MIC values are as follows:

	Zone diameter high level resistant	**Equivalent MIC**
Gentamicin 250 µg	< 14 mm (HLR)	> 500 µg/ml
Kanamycin 500 µg	< 14 mm (HLR)	> 1000 µg/ml
Streptomycin 500 µg	< 14 mm (HLR)	> 1000 µg/ml

In general we may conclude:

a) If a strain shows HLR to Streptomycin: this aminoglycoside will not show synergistic killing in combination with a penicillin (or vancomycin).

b) If a strain shows HLR to Kanamycin: this aminoglycoside and amikacin cannot be used.

c) If a strain shows HLR to Gentamicin: then the strain is HLR to all aminoglycosides, except streptomycin. Streptomycin might be useful, if the strain does not show HLR to streptomycin.

E. faecium shows intrinsic resistance towards kanamycin, tobramycin and netilmicin due to the production of the enzyme AAC (6'). Consequently there is no synergy with beta-lactams.

Quality Control

NEO-SENSITABS™	Potency	Code	***E. faecalis*** **ATCC 51299**	***E. faecalis*** **ATCC 29212**
Gentamicin	250 µg	GN 250	no zone (R)	17-23
Streptomycin	500 µg	ST500	no zone (R)	-

MH-agar, inoculum McF 0.5, incubation 35 °C 16-18 hours.

References

1) Amsterdam D.: Simple detection of high level resistance of Enterococcus faecalis to aminoglycosides. An alternative to synergy testing. The Antimicrobic Newsletter **5**, 36-38, 1988.

2) Spiegel C.A.: Laboratory Detection of High-Level Aminoglycoside Aminocyclitol Resistance in Enterococcus spp. J. Clin. Microbiol. **26**, 2270-2274, 1988.

3) Huycke M.M. et al: Bacteremia Caused by Hemolytic, High-Level Gentamicin- Resistant Enterococcus faecalis. A.A.C. **35**, 1626-1634, 1991.

4) Sahm D.F. et al: Detection of High-Level Aminoglycoside Resistance in Enterococci Other Than Enterococcus faecalis. J. Clin. Microbiol. **29**, 2595-2598, 1991.

5) Torres C. et al: Detection of aminoglycoside-penicillin synergy against Enterococcus faecium using high control aminoglycoside disks. Eur. J. Clin. Microbiol. Infect. Dis. **14**, 878-82, 1995.

FACTOR X, V, and X+V

REF No. 42511

REF No. 42611

REF No. 42711

Contain growth factors for the differentiation of ***Haemophilus*** **spp.: Hemin** (X-Factor) and **NAD** (V-Factor).

Principle of the Test

Haemophilus influenzae requires both X-Factor and V-Factor for growth, while ***Haemophilus parainfluenzae*** requires V-Factor only. Growth around the diagnostic tablets (and not on the rest of the plate) is taken as evidence of requirement for either growth factor alone or both factors together.

Procedure

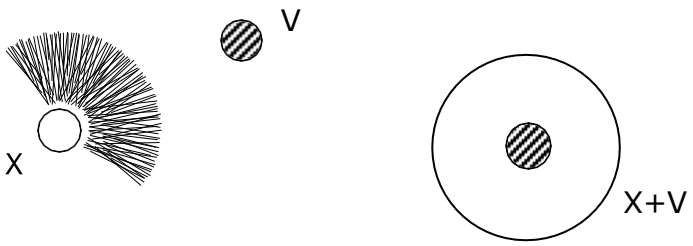
Make a suspension in saline (approx. 0.5 McFarland) of colonies from an agar plate and swab the suspension on a medium free of the two growth factors (e.g. TSA agar). Place the diagnostic tablets containing X-, V-, and X+V-Factors onto the agar; Factor X and Factor V at a distance of approx. 2 cm from each other and Factor X+V somewhat further away from these. Incubate the plate in 5-10% CO_2 at 35-37 °C for **18-24 hours**.

Reading of the Test

a) *Haemophilus influenzae*

Growth is seen only around the Factor X+V tablet and **between** the Factor X and Factor V tablets (Fig. 1).

Fig. 1

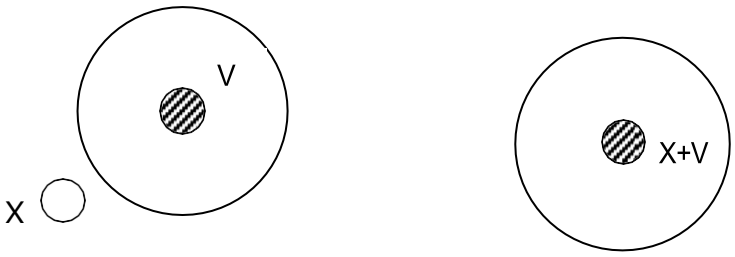


The area of growth between the Factor X and the Factor V tablets is **closer to the Factor X tablet** due to higher diffusability of V-Factor than X- Factor, giving a semicircle of growth around the X-Factor tablet. *Haemophilus influenzae* strains with very small V-Factor requirements (0.04 mg/liter or similar) may give a **full circle** of growth around the Factor X tablet.

b) *Haemophilus parainfluenzae*

Growth is seen only around the Factor V and the Factor X+V tablets (Fig. 2).

Fig. 2



The growth zones around Factor X+V are considerably larger than those seen for *Haemophilus influenzae* due to higher diffusability of Factor V compared to Factor X.

Choice of medium

The medium should be tested with known cultures of *H. influenzae* and *H. parainfluenzae* to make sure it is adequate for the test avoiding the following problems:

a) The medium lacks adequate amounts of other nutrients essential for growth of *Haemophilus* spp.

TSA-agar (e.g. BBL) has been recommended for the test allowing growth of more strains than the less nutrious Mueller-Hinton agar (Doern & Chapin, 1984). Other media may be used, but must be checked for content of X-and V- Factors (see b) and c)).

b) The medium contains hemin (X-Factor)

Haemophilus influenzae will show the reaction of a strain requiring only V-Factor and can be misidentified as *Haemophilus parainfluenzae*. Similar reactions can be seen as a result of carry-over from chocolate agar when preparing the inoculum for the test. Check with known *H. influenzae* strains to assure there is no growth around the Factor V tablet.

c) The medium contains NAD (V-Factor)

Haemophilus influenzae requires only small amounts of V-Factor (approx. 0.04 - 0.2 mg/liter (Evans et al., 1974)), and some media contain sufficient amounts for growth (e.g. from yeast extract (CASO-Agar Merck No. 5458)).

On these media *H. influenzae* gives the pattern of a strain requiring only X-Factor - growth around Factor X and Factor X+V tablets with growth zones of equal size. Small contents of V-Factor will not usually interfere with the reaction of *H. parainfluenzae* as this species requires considerably higher concentrations of V-Factor (approx. 1-5 mg/liter (Evans et al., 1974)).

These media may be used for the test if growth around the Factor X tablet is disregarded. The growth pattern around the Factor V and Factor X+V tablets will be correct.

Quality Control

Diatabs™ (Active ingredients)	
Factor V (b-Nicotinamide adenine dinucleotide sodium) **Factor X** (Hemin chloride) **Factor X + V**	*H. influenzae* ATCC 49247 *H. parainfluenzae* ATCC 7901

References

1) Doern G.V., Chapin K.C: Laboratory Identification of Haemophilus influenzae: Effects of Basal Media on the Results of the Satellitism Test and Evaluation of the Rap ID NH System. J. Clin. Microbiol. **20**, 599-601, 1984.

2) Evans N.M., Bell S.M., Smith D.D.: New Satellitism Test for Isolation and Identifica-tion of Haemophilus influenzae and Haemophilus parainfluenzae in Sputum. J. Clin. Microbiol. **1**, 89-95, 1975.

3) Evans N.M., Smith D.D., Wicken A.J.: Haemin and nicotinamide adenine dinucleotide requirements of Haemophilus influenzae and Haemophilus parainfluenzae. J. Med. Microbiol. **7**, 359-365, 1974.

4) Kilian M., Sørensen I., Frederiksen W.: Biochemical characteristics of 130 recent isolates from Haem. influenzae meningitis. J. Clin. Microbiol. **9**, 409-412, 1979.

5) Santanam, P.: A Modified Method for Differentiation of Haemophilus influenzae from Haemophilus parainfluenzae. Eur. J. Clin. Microbiol. **3**, 150-151, 1984.

6) Quentin R., Musser J.M., Mellouet M., Sizaret P.-Y., Selander R.K., Goudeau A.: Typing of Urogenital, Maternal, and Neonatal Isolates of Haemophilus influenzae and Haemophilus para-influenzae in Correlation with Clinical Source of Isolation and Evidence for a Genital Specifity of H. influenzae Biotype IV. J. Clin. Microbiol. **27**, 2286-2294, 1989.

FOSFOMYCIN 70 µg (FOSFO) Neo-Sensitabs™

REF No. 74212

We have been using Fosfomycin 70 µg Neo-Sensitabs™ for a long time in our laboratory as an aid in the identification of staphylococci. We find, in accordance with Iwantscheff (1988), that the staphylococci may be divided into three groups:

a) strains resistant to fosfomycin (*S. capitis*),

b) strains with intermediate sensitivity, and

c) the most sensitive strains.

The degree of sensitivity to fosfomycin differs for some species that are otherwise closely related, e.g. *S. saprophyticus* is considerably more resistant than the other novobiocin resistant species, *S. xylosus* and *S. cohnii*.

Procedure

Sensitivity testing is performed on Mueller-Hinton II Agar with an inoculum equivalent to McFarland 0.5. Incubation at 35-37 °C **overnight**.

Results

1) Human staphylococci

	FOSFO
a) *S. capitis* *S. capitis* ssp. ureolyticus *S. caprae*	no zone
b) *S. hominis*, *S. haemolyticus*, *S. warneri*, *S. saprophyticus*	small zone < 28 mm
c) *S. aureus*, *S. epidermidis*, *S. lugdunensis*, *S. schleiferi*, *S. xylosus*, *S. cohnii*, *S. cohnii* ssp. urealyticum, *S. simulans**	zone > 30 mm

***** *S. simulans* show growth of resistant colonies inside the inhibition zone (□□40 mm).

2) Coagulase negative mastitis staphylococci

	NOVO5	DEFRX	FOSFO	PYR (1h)	AlkP (4 h)	Remarks
S. hyicus	S (≥14 mm)	R (≤14 mm	S (≥30 mm)	0	+	
S. chromogenes	S	R	S	V	+	
S. simulans	S	R	S	+	V	
S. warneri	S	R	R (≤28 mm),	V	0	URE +
S. haemolyticus	S	R	R	+	0	URE 0
S. epidermidis	S	S (≥16 mm)	S	0	+	
S. hominis	S	S	R	0	0	
S. xylosus	R (<13 mm)	R	V	+	V	

NOVO5 = Novobiocin 5 µg Neo-S, DEFRX = Deferoxamine Diatabs™, FOSFO = Fosfomycin 70 µg Neo-S.

3) Corynebacteria and Listeria are resistant to fosfomycin, therefore Fosfomycin 70 µg Neo-Sensitabs™ may be used on blood agar plates for isolation/screening of diphtheroids (growth near the edge of the tablet).

References

1) Iwantscheff A.: In-vitro activity of fosfomycin against different Staphylococci species. J. Antimicrob. Chemother. **21**, 379-381, 1988.

2) Devriese L.A. et al: A simple identification scheme for coagulase negative staphylococci from bovine mastitis. Research in Vet. Science **57**, 240-4, 1994.

3) Foster G. et al: Staph. lutrae sp. nov., a new coagulase positive species isolated from otters. Intl. J. Syst. Bacteriol. **47**, 724-6, 1997.

4) Leung M.J.: Colony variation in Staphylococcus lugdunensis. J. Clin. Microbiol. **36**, 3096-8, 1998.

5) von Gravenitz A. et al: Coryneform bacteria in throat cultures of healthy individuals. J. Clin. Microbiol. **36**, 2087-8, 1998.

FURAZOLIDONE 50 µg (FURAZ) MUPIROCIN 10 µg (MUPIR) Neo-Sensitabs™

REF No. 74412

REF No. 75712

Furazolidone and Mupirocin are useful in the differentiation of **staphylococci** (sensitive) from **micrococci** (resistant). Besides, they are useful in the differentiation of enterococci and some coryneform bacteria.

Procedure

Sensitivity testing of staphylococci or micrococci is performed on Mueller- Hinton II Agar without blood with an inoculum equivalent to McFarland 0.5 using Furazolidone 50 µg Neo-Sensitabs™ and Mupirocin 10 µg Neo- Sensitabs. Strains that cannot grow on this agar may be tested on Mueller-Hinton II agar with added 5 % blood. Incubate at 35-37 °C **overnight**. If only one test is used, we recommend Furazolidone 50 µg Neo- Sensitabs.

Results

1) Differentiation of staphylococci from micrococci/kitococcus:

1a)	**FURAZ** and **MUPIR**
Sensitive: (S)	≥ 16 mm: **staphylococci**
Resistant: (R)	< 16 mm: **micrococci**

Above interpretation is also valid for semi-confluent growth on Iso-Sensitest, DST, PDM II and Danish Blood Agar.

1b)	**FURAZ**	**OXA**	**ADH**	**MUPI**
Staphylococcus spp.	S	V	V	S
Micrococcus spp.	R	S	0	R
Kitococcus spp.	R	R	+	

2) Differentiation of enterococci (CAT 0, PYR+, LAP+, BE+, VP+)

2a)	**MUPIR**	**FURAZ**	**NOVO5**
Enterococcus faecalis	R (NZ)	S	R (<13 mm)
Enterococcus faecium	S	R (NZ)	S (≥14mm)
Other enterococci	S^R	S	S

Most current human enterococci

2b)	ADH	MAN	SOR	ARA	MOT	TEL	Fura		Remarks
E. avium	0	+	+^{0}	+	0	S	V	V	RAF0
E. raffinosus	0	+	+	+^{0}	0	S	·	·	RAF+
E. faecalis	+	+	+	0	0	R	S	R	
E. faecium	+	+^{0}	V	+	0	V	R	S	
E. gallinarum	+	+^{0}	V	+	+	S^{R}	S	S	Pigm0, XYL®+
E. casseliflavus	+	+^{0}	·	+	+	S	S	S	Pigm+
E. durans	+	0	0	0	0	S	R	S	αGAL0
E. hirae	+	0	0	0	0	S	R	S	αGAL+

MUPIR = Mupirocin 10 µg Neo-S, FURAZ = Furazolidone 50 µg Neo-S, NOVO5 = Novobiocin 5 µg Neo-S, MOT = motility, NZ = no zone, PIGM = pigment, R^{S} = Most strains resistant, XYL^{R} = Rapid Xylose Diatabs™ (incub. 2 h at 37 °C, McF 3) (9), α-GAL = Alpha-Galactosidase Diatabs™, OXA = Oxacillin Neo-S, ADH = Arginine Dihydrolase Diatabs™, ARA = l-Arabinose, MAN = Mannitol Diatabs™, SUC = Sucrose Diatabs™, TEL = Tellur 500 µg Diatabs™, ß-MAN = Beta-mannosidase, NAG = N-acetyl glucosaminidase Diatabs™, VP = Voges-Proskauer Diatabs™,

3) Coryneform bacteria

	FURAZ	O/129	LAP
C. minutissimum	S (zone)	S	+
C. amycolatum	R (no zone)	R	0

O/129 = O/129 150 µg Diatabs™, LAP = Leucine Aminopeptidase.

4) Throat cultures

	BaL	MUPIR	PYR
Arcanobact. haemolyticum	R	R	0
Streptococcus pyogenes (A)	S	S	+
Streptococcus group C/G	R(V)	S	0

BaL = Bacitracin low 0.4 U N.D. (S > 15 mm), MUPIR = Mupirocin 10 µg Neo-S (R = no zone), PYR = Pyrrolidonyl Aminopeptidase Diatabs™

5) Agents of Zoonotic infections. Differentiation of streptococci, corynebacteria and listeria (11)

	MUPI	VANCO	FOSFO	PYR	PRO	PGUA	H_2S	Haem.
Arcanob. pyogenes	R	S	S	+	+	+	0	Beta
Strept. suis	S	S	S	+⁰	.	+´⁰	0	Alpha
Erysipel. rhusiopathiae	.	R	S	+⁰	V	.	+	CAT 0
Streptococci spp.	S	S	S	0⁺	.	0⁺	.	
Corynebacteria spp.	R	S	R	0⁺	+⁰	0⁺	.	α-MAN 0
Listeria spp.	R	S	R	0	0	0	.	α-MAN +

MUPI = Mupirocin Neo-S (S ≥ 16 mm and R < 16 mm), VANCO = Vancomycin Neo-S (S ≥ 16 mm and R < 16 mm), FOSFO = Fosfomycin Neo-S (S ≥ 16 mm and R < 16 mm), PYR = Pyrrolidonyl Aminopeptidase Diatabs™, PRO = Proline Aminopeptidase Diatabs™, PGUA = Beta-Glucuronidase Diatabs™, H_2S = Hydrogen sulphide production. Haem = Haemolysis.

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2) De la Fuente et al: Comparison of methods for routine separation of coagulase negative staphylococci from micrococci isolated from sheep. Comp. Immunol. Microbiol. Infect. Dis. **9**, 347-353, 1986.

3) Casals J.B., Pringler N.: The value of 3 tests in the identification of staphylococci: Pyrrolidonyl aminopeptidase (PYR) and Susceptibility towards Polymyxins and Furazolidone. Staphylococci Symposium, Society for Applied Bacteriology, Edinburgh, July 1989.

4) Casals J.B., Pringler N.: Identification of staphylococci using a combination of chromogenic substrates and sensitivities towards Furazolidone, Novobiocin and Colistin. Workshop on Pathogenesis of Wound and Biomaterial-Associated Infections, Lund University, 1989.

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9) Chen D. K. et al: Evaluation of d-Xylose and 1% Methyl D-glucopyranoside fermentation tests for distinguishing Ent. gallinarum from Ent. faecium. J. Clin. Microbiol. **38**, 3652-5, 2000.

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GLYCOSIDASES

General description

The chromogenic glycosidases tests are based upon enzymatic release of yellow-coloured nitrophenol from the substrates. Because the tests detect preformed enzymes non-growing suspensions can be used, and the tests are thus applicable also to microorganisms that do not grow in conventional test media. The tests are rapid and relatively inexpensive.

Range

The range of Glycosidase Diatabs™ comprises:

Beta-N-Acetylglucosaminidase	(NAG)	(50011)
Alpha-Fucosidase	(α-FUC)	(50111)
Beta-Fucosidase	(ß-FUC)	(59911)
Alpha-Galactosidase	(α-GAL)	(50211)
Beta-Galactosidase	(ONPG)	(50311)
Alpha-Glucosidase	(α-GLU)	(50411)
Beta-Glucosidase	(ß-GLU)	(50511)
Beta-Glucuronidase	(PGUA)	(50611)
Alpha-Mannosidase	(α-MAN)	(50711)
Beta-Xylosidase	(ß-XYL)	(50811)

Procedure

Prepare a dense "milky" bacterial suspension (at least McFarland No. 4) from the strain to be tested in 0.25 ml saline in a tube. Add one diagnostic tablet and close the tube. Incubate at 35-37 °C for **4 hours** or **overnight**.

Reading of the tests

Positive reaction: **Yellow**

Negative reaction: Colourless

With strains that produce a yellow pigment (e.g. *Enterob. agglomerans*, Flavobacterium, Xanthomonas) or a red pigment (Serratia) use the bacterial suspension without the tablet (negative control) as control of colour, in order to facilitate the readings.

The tests are useful in identification of a wide variety of bacterial strains, including Enterobacteriaceae, non-fermenters, staphylococci, streptococci, anaerobes, neisseria, and haemophilus.

References General

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11) Rautio M. et al: Characteristics of an unusual anaerobic pigmented gram negative rod isolated from normal and inflamed appendices. Clin. Infect. Dis. **25**, Suppl. 2, S107-S110, 1997.

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BETA-N-ACETYLGLUCOSAMINIDASE (ß-NAG)

REF No. 50011

Results

1) Streptococci (*milleri*)

	NAG
S. intermedius	+
S. anginosus/constellatus	0

2) Actinomyces

Most strains are: Vanco S, Col R, Metro R^S, Cipro R, Kana S.

	NAG	ONPG	PZA
A. europaeus	0	+	0
A. radingae	+	+	+
A. turicensis	0	0	0

3) Identification of *C. albicans* (4 h)

	NAG(24h)	PRO	42 °C	XYL	2h αGLU
Candida albicans	$+^0$	+	+	+	+
C. dublinensis	+	+	0	0	0
A) *Candida* spp.	0	+	·	·	V
B) *Candida* spp.	0	0	·	·	0^+

NAG (24 h) needs overnight incubation

where A) comprises: *C. guilliermondii*, *C. lipolytica*, *C. lusitaniae*, *C. norvegensis*, *C. parapsilosis*, *Tor. Candida*.

where B) comprises: *C. glabrata*, *C. krusei*, *C. pseudotropicalis*, *C. rugosa* (NAG 0^+), *C. tropicalis* (NAG 0^+).

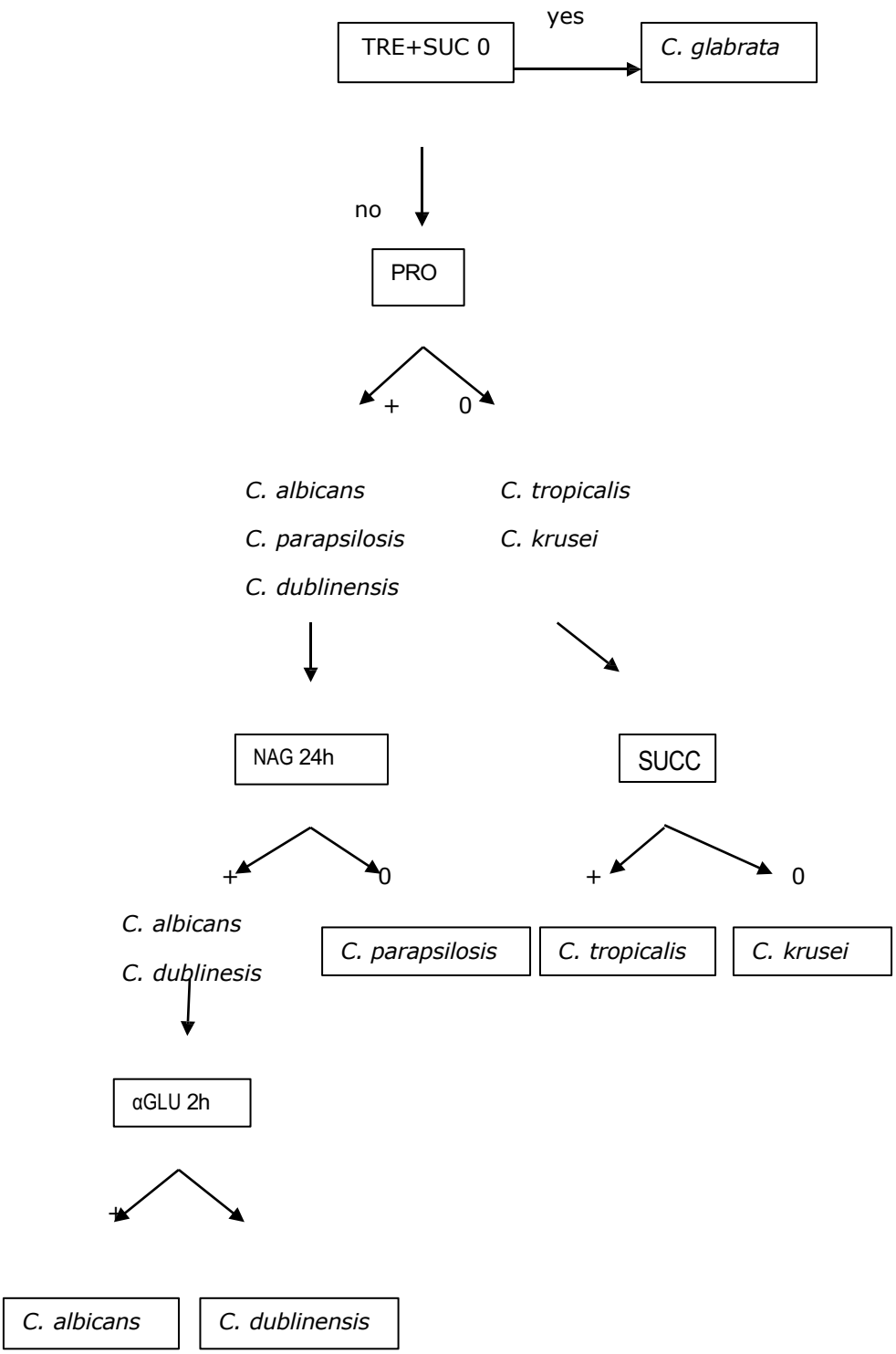
NAG = Beta-N-Acetylglucomaninidase Diatabs™, ONPG = ONPG Diatabs™, PZA = Pyrazinamidase Diatabs™, PRO = Proline Amino-peptidase Diatabs™, 42 °C = Growth at 42 °C in Sabouraud Glucose Agar, XYL = d-Xylose Diatabs™

4) Differentiation of most current *Candida* species (4 hours)

	Alk P	NAG (24h)	PRO	TRE	SUC	CYC	42 °C	2h αGLU
C. albicans	0	+	+	V	+^{0}	R (no zone)	+	+
C. glabrata	0	0	0 (V)	+	0	S	·	0
C. bracarensis	·	0	0	+	0	R	+	·
C. krusei	+	0	0	0	0	S	·	0
C. parapsilosis	0	0	+	0	+^{0}	R^{S}	·	+
C. tropicalis	+	0^{+}	0	V	+^{0}	R^{S}	·	+
C. dublinensis	·	+$^{´0}$	+	V	+^{0}	·	0	0

TRE = Trehalose Diatabs™, SUC = Sucrose Diatabs™, CYC = Cycloheximide Diatabs™ (S ≥ 25 mm, R < 25 mm). AlkP
= Alkaline Phosphatase Diatabs™, 42 °C = Growth at 42 °C in Sabouraud Glucose Agar, 2h αGLU = Alpha- Glucosidase Diatabs™ (incubation 2 hours). NAG is read after 24 hours incubation (if negative, after 4 hours)

Algorithm


TRE+SUC 0
yes
C. glabrata
no
PRO
+
0
C. albicans
C. parapsilosis
C. dublinensis
C. tropicalis
C. krusei
NAG 24h
SUCC
+
0
+
0
C. albicans
C. dublinesis
C. parapsilosis
C. tropicalis
C. krusei
αGLU 2h
+
C. albicans
C. dublinensis

5) Differentiation inside the *Clostridium clostridioforme* group (3,4)

	NAG	ONPG	RAF	IND	spores	Remarks
C. clostridioforme	0	+	+	0	+	MOT+
C. bolteae	0	0	70	0	+	
C. hathewayi	+	V	+	0	+	
C. citroniae	0	0	0	+	+	
C. aldenense	0	+⁰	+	+	+	
Moryella indoligenes	·	0	0	+	0	
Robinsoniella peonensis	+	+	·	0	+	PGUA+, αFUC+, MOT 0

RAF = Raffinose.D.T, IND = Indole Diatabs™

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Beta-N-Acetylglucosaminidase (p-Nitrophenyl-N-acetyl-β-D-glucosaminide)	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 25923

References (ß-NAG)

1) Jousimies-Somer H.R. et al: Anaerobic gram-negative bacilli and cocci. Manual of Clin. Microbiology 5th Ed. ASM, 538-552, 1991.

2) Niimi K. et al: Destinguishing Candida species by ß-N-acetylhexosaminidase activity. J. Clin. Microbiol. **39**, 2089-97, 2001.

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4) Warren Y.A. et al: Clostridium aldenense and Cl. citroniae sp nov isolated from human clinical infections. J. Clin. Microbiol. 44, 2416-22, 2006.

BETA-FUCOSIDASE (ß-FUC)

REF No. 59911

Results

1) Streptococcus "milleri" anginosus group (ADH +, CAT 0, ESC +, VP +, MAN 0, PYR 0, SOR 0)

	ß-FUC	NAG	ß-GLU	RAF	α-GLU	Remarks
S. anginosus	0	0	+	V	0+	αGAL+, AlkP+
S. constellatus	0	0	0	0	+0	
S. constellatus subsp. *Pharyngis*	+	+	+0	0	·	
S. intermedius	+	+	V	0+	+	
S. sinensis	·	0	+	0	·	BE+. αGAL0,AlkP0

NAG = Beta-N-Acetylglucosaminidase Diatabs™, ß-GLU = Beta-Glucosidase, RAF = Raffinose Diatabs™, α-GLU = Alpha-Glucosidase Diatabs™

2) Differentiation of Group C and G beta-haemolytic streptococci

	VP	PGUA	ß-GLU	ß-FUC	LACT	SORB	HIP	TRE
S. anginosus (ACG)	+	0	+	0		0		+0
S. constellatus (ACG)	+	0	0	0		0		·
S. dysgalactiae * subsp. *Equisimilis (ACG)*	0	+	V	0		0		+
S. constellatus subsp. *pharyngis*	+	0	+0	+		0		·
S. equi subsp. *zooepidemicus (C)*	0	+	V	0	+	+	0	0
S. canis (G)	0	0	V	0		0		
S.equi (C) *subsp. equi*	0	+	+0	0	0	0	0	
S. equi subsp ruminatorum (C)	0	+	0	0	+	V	+	

* may be alpha-haemylytic sometimes.

S. canis is PYRV, CAMP+^{0}, ONPG+ and αGAL+^{0}.

VP = Voges Proskauer Diatabs™, PGUA = Beta-Glucuronidase Diatabs™, ß-GLU = Beta-Glucosidase Diatabs™, ß-FUC Beta-Fucosidase Diatabs™, SORB = Sorbitol Diatabs™, TRE = Trehalose Diatabs™

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Beta-Fucosidase (p-Nitrophenyl-ß-D-fucopyranoside)	*S. intermedius* ATCC 27335	*E. coli* ATCC 25922

References

1) Whiley R.A. et al: Phenotypic differentiation of S. intermedius S. constellatus and S. anginosus strains within the "S. milleri group". J. Clin. Microbiol. **28**, 1497-1501, 1990.

2) Whiley R.A. et al: A study of small-colony, beta-haemolytic, Lancefield group C streptococci within the anginosus group: description of S. constellatus subsp. pharyngis subsp. nov., associated with the human throat and pharyngitis. I.J.S.E.M. **49**, 1443-9, 1999.

3) Gray T.: Streptococcus anginosus group: clinical significance of an important group of pathogens. Clin. Microbiol. Newslettter **27**, 155-9, 2005.

ONPG - Beta-Galactosidase (ONPG)

REF No. 50311

Results

1) Actinobacillus/Pasteurella

	ONPG	URE	IND
Actinobacillus spp.	+	+	0
Pasteurella spp.	0	0^{+}	+

2) Actinomyces

Most strains are: Vanco S, Col R, Metro R^{S}, Cipro R, Kana S.

	ONPG	NAG	PZA
A.. europaeus	+	0	0
A. radingae	+	+	+
A. turicensis	0	0	0

ONPG = ONPG Diatabs™, URE = Urease Diatabs™, IND = Indole Diatabs™, NAG = Beta-N-Acetylglucosaminidase Diatabs™ and PZA = Pyrazinamidase Diatabs™

3) HACEK group and miscellaneous gram negative rods/cocobacilli (Capnocytophaga, *Pasteurella* spp.)

	γ-GLU	OXI	CAT	SUC	α-GLU	ONPG	TRYP	IND	NIT	Remarks
*Aggregatibacter aphrophilus**	+	V	0	+	$+^{0}$	+	0	0	+	
A. actinomycetemcomitans	+	0^{+}wk	+	0	0	0	0	0	+	LAP +
Cardiob. hominis	+	+	0	+	0	0	+	wk	0	MAN +, TTR +,ODC 0
Cardiob. valvarum	·	+	0	V	+	0	+	$+^{0}$	0	MAN 0, TTR +
Eikenella corrodens	0	+	0	+	0	+	0	0	+	LDC $+^{0}$, ODC +, PRO +
Kingella spp.	0	+	0	V	0	0	·	V	0^{+}	Col R

Capnocytophaga spp.	·	0	0	+	+	$+^{0}$	$+^{0}$	0	$+^{0}$	
Capn. canimorsus	·	+ wk	+	0	+	+	+	0	0	α-FUC +, ADH +
Capn. cynodegmi	·	+	+	+	+	+	+	0	+	
Past. multocida	0	+	+	+	V	0	0	$+^{0}$	+	ODC $+^{0}$, PRO 0
Mannh. haemolytica	·	+	$+^{0}$	+	·	$+^{0}$	0	0	+	ODC 0, α-FUC +

γ-GLU = Gamma-Glutamyl Aminopeptidase Diatabs™, OXI = oxidase, CAT = catalase, SUC = Sucrose Diatabs™, α-GLU = Alpha-Glucosidase Diatabs™, TRYP = Trypsin Diatabs™, IND = Indole Diatabs™, NIT = Nitrate reduction, ALA = Porphyrin Diatabs™, Col = Colistin 10 µg Diatabs™(S ≥ 13 mm, R ≤ 10 mm), α-FUC = Alpha-Fucosidase Diatabs™

* *Aggregatibacter aphrophilus* cover the previous *H. aphrophilus* and *H. paraphrophilus*.

4) Differentiation of *Actinobacillus* spp.

Most strains are: URE +, ONPG +, NO_3, +, ADH 0, ODC 0, IND 0, O/129 S.

	OXI	**CAT**	**αGAL**	**αGLU**	**ßXYL**	**ßGLU**	**SOR**	**TRE**	**MAN**	**Remarks**
Actinobacillus hominis	+	0	+			V	0	+	+	
A. equuli ssp. *equuli*	V	+wk	+	+	+	0	0	+	+	β-XYL+
A. equuli	+	+wk	+	V	$+^{0}$	$+^{0}$	V	+	0^{+}	ß-haem $+^{0}$
ssp. *haemolyticus* (B-11)										
A. lignieresii	$+^{0}$	$+^{0}$	0	0	0	0	0	0	+	LACT +
A. pleuropneumoniae	0^{+}	V	0			V	0	0	+	LACT 0
A. suis	$+^{0}$	+	+			+	0	+	0	
A. capsulatus	+	+	+			$+^{0}$	+	+	+	
A. Bisgaard taxon 8	+	+	+			0	0	0	+	
A. arthritidis (B-9)	+	+	+	0	0	0	+	0	+	
A. genomospecies 2	+	+	+	0	V	0	0	0	+	
A. ureae	0^{+}	V	0			0	0^{+}	0	+	ONPG 0, URE $+^{R}$
"Pasteurella pneumotropica"	+	+	V			0	0	V	0	IND +, ODC +

OXI = Oxidase Diatabs™, CAT = Catalase, αGAL = Alpha-Galctosidase Diatabs™, αGLU = Alpha-glucosidase Diatabs™, ßXYL = Beta-Xylosidase Diatabs™, ßGLU = Beta-Glucosidase Diatabs™, SOR = Sorbitol Diatabs™, TRE = Trehalose Diatabs™, MAN = Mannitol Diatabs™, ß-haem = beta haemolysis, LACT = Lactose Diatabs™, URE = Urease Diatabs™, $+^{R}$ = rapidly positive, IND = Indole Diatabs™, ODC = Ornithine Decarboxylase Diatabs™

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
ONPG (Beta-Galactosidase) (o-Nitrophenyl-β-D-Galactopyranoside)	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315

References (ONPG)

1) Bruun B., Ursing J. Phenotypic charaterization of Flavobacterium meningosepticum strains identified by DNA-DNA hybridization. Acta path. microbiol. immunol. scand. Section B, **95**, 41-47, 1987.

2) Ashhurst-Smith C. et al: Actinobacillus equuli septicemia: an unsual zoonotic infection. J. Clin. Microbiol. **36**, 2789-90, 1998.

3) Friis Møller A. et al: Clinical significance and taxonomy of Actinobacillus hominis. J.Clin. Microbiol. **39**, 930-5, 2001.

4) Christensen H. et al: Final classification of Bisgaard taxon 9 as A. Actinobacillus arthritidis sp. nov. and recognition of a novel genomospecies for equine strains of A. lignieresii. IJSEM **52**, 1239-46, 2002.

5) Christensen H. et al: Raclassification of equine isolates previously reported as A. equuli, variants of A. equuli, A. suis or Bisgaards taxon 11 and proposal of A. equuli ssp. equuli ssp. nov. and A. equuli ssp. haemolyticus ssp. nov. IJSEM **52**, 1569-76, 2002.

BETA-GLUCURONIDASE (PGUA)

REF. No. 50611

Beta-Glucuronidase (PGUA) Diatabs™ are useful in the presumptive identification of ***Escherichia coli*.** As *E. coli* is the ethiological agent of approx. 80 % of urinary tract infections, a simple, specific, rapid and accurate method for its identification is very useful.

Procedure

Prepare a dense bacterial suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one PGUA tablet and close the tube. Incubate at 35-37 °C for **4 hours** (or **overnight**).

Reading of the test

Positive reaction **Yellow**

Negative reaction: Colourless

Approx. 94 % of *E. coli* are positive for the PGUA test. Among the other Enterobacteriaceae only some Shigella and Salmonella (approx. 30 %) are found positive. Strains of *Citrob. freundii* and *Enterobacter cloacae* have been found positive in uncommon cases.

Results

1) Enterobacteriaceae

	PGUA
E. coli	94
Salmonella spp.	V
Shigella spp.	V
Other	0

2) *Arcanobacterium haemolyticum* biotypes

	PGUA	**SUC**	**Infection**
A. haemolyticum smooth	0	41	wounds
A. haemolyticum rough	97	0	respiratory

PGUA = Beta-Glucuronidase Diatabs™, SUC = Sucrose Diatabs™

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Beta-Glucuronidase (PGUA) (p-Nitrophenyl- ß -D glucuronic acid)	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 13883

References (PGUA)

1) Dibb W.L., Bottolfsen K.L.: Evaluation of Rosco diagnostic beta glucuronidase tablets in the identification of urinary isolates of Escherichia coli. Acta Path. Microbiol. Scand. Sect.B. **92**, 261 264, 1984.

2) Hansen W., Yourassowsky E.: Detection of beta-glucuronidase in lactose-fermenting members of the family Enterobacteriaceae and its presence in bacterial urine cultures. J. Clin. Microbiol. **20**, 1177, 1179, 1984.

3) Pérez J.L., Berrocal C.I., Berrocal L.: Evaluation of a commercial beta-glucuronidase test for the rapid identification of Escherichia coli. J. Applied Bacteriol. **61**, 541-545, 1986.

4) Casals J.B., Pringler N.: Rapid Identification of E. coli with a Double Test Tablet: Beta Glucuronidase (PGUA)/Indole". 4th European Congress of Clinical Microbiology, Nice, 1989, poster 514.

5) Domínguez A., Alcaide F., Pulido A., Ayats J., Pérez J.L., Martín R.: Use of a Commercial Double-Test Tablet (Rosco PGUA/Indole) for Screening of Escherichia coli. Diagn. Microbiol. Infect. Dis. **15**, 291-294, 1992.

6) Vandepilte J. et al: Basic laboratory producedures in Clinical Bacteriology WHO Geneva, page 35-36 and 109, 1991.

7) Carlson P. et al: Biotypes of Arcanobacterium haemolyticum. J. Clin. Microbiol. **32**, 1654-7, 1994.

BETA-XYLOSIDASE (ß-XYL)

REF No. 50811

Results

1) Acinetobacter

	ß-XYL	γGLU
Acinetobacter baumaniii /calcoaceticus	$+^0$	$+^0$
A. lwoffi	0	0

2) Propionibacteria, Metro I/R

	ß-XYL	ONPG	Remarks
Propionibacterum acnes	0	+	(CAT +, IND +)
Propionibacterium. avidum	+	+	
P. granulosum	0	0	
P. propionicum	0	$+^0$	(CAT 0, IND 0)

3a) Enterobacteriaceae

	ß-XYL	PYR	αGLU
Klebsiella spp.	+	+	0^+
Enterobacter spp.	+	+	0^+
Yersinia spp.	V	$+^0$	0
Citrobacter spp.	0	+	0
Serratia spp.	0	+	V
Citrobacter amalonaticus	V	+	·
Serratia rubidaea	V	+	·
Other enterobacteriaceae	0	0	0^+
Cronobacter sakazakii	·	·	+

3b) Klebsiella/Enterobacter/Serratia

	PYR	ß-XYL	ODC
Klebsiella spp.	+	+	0
Enterobacter spp.	+	+	+
Serratia spp.	+	0	+

4) Capnocytophaga (PYR +, TRYP +, OXI 0, CAT 0)

	ß-XYL	NAG
Capnocytophaga gingivalis	0	0
Capnocytophaga sputigena	+	$+^{0}$
Capnocytophaga ochracea	0	+

β-XYL = Beta-Xylosidase Diatabs™, ONPG = ONPG Diatabs™, CAT = catalase, IND = Indole Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, NAG = Beta-N-Acetylglucosaminidase Diatabs™, γ-GLU = Gamma-Glutamyl Aminopeptidase Diatabs™ OCD = Ornithine Decarboxylase D.T, αGLU=Alpha Glucosidase Diatabs™

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Beta-Xylosidase (p-Nitrophenyl ß-D-xylopyranoside)	*K. pneumoniae* ATCC 13883	*E. coli* ATCC 25922

References (ß-XYL)

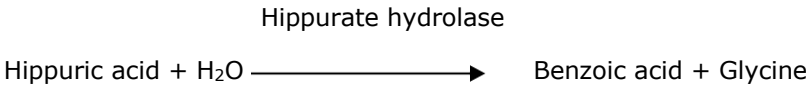
1) Jousimies-Somer H. et.al: Bacteroides, Porphyromonas, Prevotella, Fusobacterium and other anaerobic gram-negative bacteria. Manual Clinical Microbiology 6th Ed., 603-618, 1995.

2) Murray P.R., Citron D.M.: General processing of specimens for anaerobic bacteria. Manual Clinical Microbiology 5th Ed., 488-500, 1991.

HIPPURATE HYDROLYSIS (HIP)

REF No. 56711

Diagnostic Tablets for determining the ability of bacterial strains to hydrolyze hippurate by the action of the enzyme hippurate hydrolase. The tablets contain sodium hippurate which is split into benzoic acid and the amino acid glycine. The latter is detected in the test by addition of Ninhydrin solution.



Procedure

Prepare a dense suspension of the strain (at least McFarland No. 4) to be tested in 0.25 ml saline in a tube. Add one Hippurate Hydrolysis Diagnostic Tablet, close the tube and incubate for **4 hours** or **overnight** at 35-37 °C.

Reading of the test

Positive reaction: **Deep purple - blue**

Negative reaction: Colourless, light yellow or occasionally a faint tinge of purple

Do not reincubate longer than 10 minutes as false positives may result. Do not use reagents other than ninhydrin to make the colour reaction. The test is useful in the presumptive identification of Group B streptococci, *Gardnerella vaginalis*, and *Campylobacter jejuni*.

Results

1a) *Streptococcus* spp.

	HIP
Group B streptococci	+
Other beta-haemolytic Streptococci (except group D)	0

For detection of group B streptococci prenatal from vaginal/rectal specimens. After overnight incubation in selective broth, were subbed to TSA+5% sheep blood. A 10 µg gentamicin disk is placed in the second quadrant of the plate. Incubate overnight. Group B streptococci will show a narrow zone of beta-haemolysis and Group B will grow near the gentamicin disk, while enterococci will grow further away from the disk (10).

1b) Streptococci from subclinical mastitis

	HIP	ESC	PGUA	PYR	CAMP
S. agalactiae (B)	+	0	V	0	+
S. dysgalactiae	0	0^{+}	+	0	0
subsp. *dysgalactiae* (C)					
S. uberis (E, P, U, G)	$+^{0}$	+	+	+	0^{+}
S. parauberis	$+^{0}$	+	0	+	0
S. canis (G)	0	$+^{0}$	0^{+}	V	+

ESC = Esculin Hydrolysis Diatabs™, PGUA = Beta-Glucuronidase Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, CAMP = CAMP reaction.

2a) *Campylobacter* spp. (11) See also 2b)

	HIP
Campylobacter jejuni	+
Helicobacter westmeadii	+
Other campylobacter / Helicobacter	0

2b) Differentiation of enteropathogenic Campylobacters / *Arcobacter butzleri*

	HIP	IAC	CAT	CLOTN	25 °C	COL	Remarks
C. coli	0	+	+	R	0	R	
C. jejuni	+	+	$+^{0}$	R^{S}	0	R	
C. lari	0	0	+	R	0	R	H_2S+
C. upsaliensis	0	+	0 wk	S	0	R	
C. fetus	0	0	+	S	+	R	
Arcobacter butzleri	0	$+^{0}$	V	R	+	S	
C. insulaenigrae	0	0	+	R	0	R	42°C+,NO_3+,H_2SO

IAC = Indoxyl Acetate Diatabs™, CAT = catalase, CLOTN = Cephalothin Neo-S (S ≥ 16 mm, R < 16 mm), 25 °C = Growth at 25 °C, COL = Colistin.

For Campylobacter it is important to harvest bacteria from blood-agar (TSA or Columbia agar + blood) and to use a high inoculum. Nakari et al (11) found that an inoculum corresponding to McFarland n^r6 to n^r10, gives the best results and eliminates false negatives.

After incubation add 5 drops of **Ninhydrin 3.5% sol.** (91731), close the tube and **reincubate** for **10 minutes** at 35-37 °C. Read within 5 minutes.

Please notice that a certain amount of *C. jejuni* are HIP negative and can only be detected using molecular methods (11).

3) *Gardnerella vaginalis* and *Atopobium vaginae*

CAT 0, OXI 0

	HIP	SPS	Remarks
Gardnerella vaginalis	+	S	(≥10 mm), PRO +
Bifidobacteria	0	R	
Lactobacilli/dipftheroids	V	R	
Atopobium vaginae	.	R	PRO +, ADH +, LAP +, Metro R, Vanco S

4) Nutritionally variant streptococci (NVS) = *Abiotropia* spp. and *Granulicatella* spp and *Helcococcus* spp (most PYR+)

	HIP	PGUA	α-GAL	ADH	PYR	ONPG
A. defectiva	0	0	$+^0$	0	+	+
G. adiacens	0	$+^0$	0	0	+	0
G. elegans	+	0	0	+	+	0
G. baldenopterae	0	0	0	+	.	.
Helcococcus kunzii	0	0	0	0	+	+
Helcococcus sueciensis	0	0	0	0	0	+

5) Differentiation of *Facklamia* spp.

	HIP	ADH	SUC	TRE
Facklamia hominis	+	+	V	0
Facklamia ignava	+	V	+	0^{+}
Facklamia languida	0	0	0	+
Facklamia sourekii	+	0	+	+
Facklamia tabacinasalis	0	0	+	+

HIP = Hippurate Hydrolysis Diatabs™, PGUA = Beta-Glucuronidase Diatabs™, α-GAL = Alpha-Galactosidase Diatabs™, ADH = Arginine Dihydrolase Diatabs™, SUC = Sucrose Diatabs™, TRE = Trehalose Diatabs™

6) Catalase negative cocci from milk (8)

	LAP	PYR	HIP	INU	RAF
S. uberis	+	+	+	$+^{0}$	0
S. bovis	+	0	0	V	+
S. dysgalactiae	+	0	0	0	0
Aerococcus spp.	0	+	-	-	-
Enterococcus/lactococcus	+	+	V	0	V

LAP = Leucine Aminopeptidase Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, HIP = Hippurate Hydrolysis Diatabs™, INU = Inulin Diatabs™, RAF = Raffinose Diatabs™

7) Differentiation of *Mobiluncus* spp.

	HIP	ADH	ONPG	α-FUC	α-GLU
Mobiluncus curtisii	+	+	$+^{0}$	0	+
Mobiluncus mulieris	0	0	0	V	+

ADH = Arginine Dihydrolase Diatabs™, α-FUC = Alpha-Fucosidase Diatabs™, α-GLU = Alpha-Glucosidase Diatabs™

8) Presumptive ID of Legionella pneumophila (Motile, URE neg, NO_3 neg, galatinase+)

Use colonies isolated on buffered charcoal-yeast extract agar (BCYE), after 24-96 hours growth.

Use a very dense suspension in 0.25 ml saline. Incubate overnight at 35-37°C.

Thereafter add 5 drops ninhydrin solution, close the tube and mix the contents well and observe for color development within 20 minutes.

	HIP
Legionella pneumophila	+
L. micdadei, bozemanii, dumoffii	0

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Hippurate Hydrolysis (Hippuric acid Sodium-salt)	*S. agalactiae* ATCC 12386	*S. pyogenes* ATCC 12344

References

1) Bastida Vilá M.T. et al: Gardnerella vaginalis bacteremia in an adult male. Eur. J. Microbiol. Infect. Dis. **16**, 400-1, 1997.

2) Roggenkamp A. et al: Abiotropia elegans sp. nov. a possible pathogen in patients with culture negative endocarditis. J. Clin. Microbiol. **36**, 100-4, 1998.

3) Sorlin P. et al: Recurrent "Flexispira rappini" bacteremia in an adult patient undergoing hemodyalisis: case report. J. Clin. Microbiol. **37**, 1319-23, 1999.

4) Sato S. et al: Abiotropia elegans strains comprise 8% of the nutririonally variant streptococci isolated from the human mouth. J. Clin. Microbiol. **37**, 2553-6, 1999.

5) Lawson P.A. et al: Facklamia languida sp. nov. isolated from human clinical specimens. J. Clin. Microbiol. **37**, 1161-4, 1999.

6) Christensen J.J. Facklam R.R.: Granulicatella and Abiotrophia species from human clinical specimens. J. Clin. Microbiol. **39**, 3520-3, 2001.

7) On S.L.W.: Identification methods for Campylobacters, Helicobacters and related organisms. Clin. Microbiol. Reviews **9**, 405-22, 1996.

8) Fortin M et al: Identification of catalase negative, non-beta-haemolytic gram-positive cocci isolates from milk samples. J. Clin. Microbiol. **41**, 106-109, 2003.

9) Hoyles L et al: Transfer of members of the genus Falcivibrio to the genus Mobiluncus and amended description of the genus Mobiluncus. System. Appl. Microbiol. **27**, 72-83, 2004.

10) Kornherr P. et al: Comparison of culture media and methods used for the detection of Group B streptococcus from prenatal vaginal/rectal specimens. ASM meeting, presentation C-133, June 2008.

11) Nakari U.M. et al: Correct identification and discrimination between Campylobacter jejuni and C. coli by a standardized hippurate test and species specific PCR. Eur. J.Clin Microbiol. Infect Dis **27**, 513-518, 2008.

INDOXYL ACETATE (IAC)

REF No. 59551

Diagnostic tablets that are useful in the identification of *Campylobacter* spp. *C. jejuni*, *C. coli* and *C. upsaliensis* are positive while other *Campylobacter* spp. are negative. The related species *Arcobacter cryaerophilus*, *Arcobacter butzleri,* and *Helicobacter fennelliae* and occasionally Helicobacter cinaedi (weak pos.) also give a positive reaction while *Helicobacter pylori* is negative. Indoxyl Acetate is packed in vials of 15 tablets. **Store at 2-8°C.**

Principle

The diagnostic tablets are used to detect the presence of acetate esterase in microorganisms. Organisms possessing acetate esterase activity hydrolyze indoxyl acetate into acetic acid and free indoxyl. Free indoxyl reacts with oxygen, which results in a blue/green colour (positive).

Procedure

Prepare a dense "milky" suspension equivalent to at least McFarland No.4 from freshly-grown colonies into 0.25 ml saline in a small tube. Add one Indoxyl Acetate Diagnostic Tablet and close the tube. Incubate at 37 °C in ambient air for **4 hours** or **18-24 hours**.

Reading of the test

Positive reaction: **Blue, green sediment**

Negative reaction: Colourless, slightly coloured supernatant (sediment not blue)

Results

1) Campylobacter/Helicobacter

Most strains are: OXI +, CAT +.

	IAC	γGLU
Campylobacter jejuni	+	V
Campylobacter coli	+	0
Campylobacter upsaliensis	+	·
Helicobacter fennelliae	+	0
H. salomonis/bizzozeroni	+	+

Campylobacter lari	0	·
Helicobacter pylori	0	+
H. felis/cynogastricus	0	+
H. suis	0	+

2) *Helicobacter* spp. isolated from <u>human blood</u> (CAT +, OXI +, PZA 0, Growth 25 °C 0, COLR)

	Susceptibility								
	IAC	**AlkP**	**NO_3**	**URE**	**GLU**	**NAL**	**CLTN**	**42°C**	**Remarks**
Helicobacter spp. VA,BC	+	+	0	0	·	S(>16mm)	R	0	
Helicobacter westmeadi	0	+	+	V	·	S	R	0	HIP +
Helicobacter cinaedi	0wk	0	+	0	0	S	V/I	0	
Helicobacter mainz	0	0	0	0	·	R	S(>16mm)	0	
Helicobacter fennelliae	+	+	0	0	0	S	S	0	
Flexispira rappini (CAT 0)	0	0	0	$+^R$	+	R	R	+	
Flexispira like (CAT +)	0	+	0	+	·	R	R	0	
Helicobacter canis	+	+	0	0	+	S	R/I	+	CAT <u>0</u>
Helicobacter pullorum	0	0	+	0	+	R	S	+	COL S, Oxgall R,
Helicobacter macacae	+	0	0	0	0	R	R	+	CAT Weak
Helicobacter suis	0	+	0	+	+			0	

IAC = Indoxyl Acetate Diatabs™, HIP = Hippurate Hydrolysis Diatabs™, NO_3 = Nitrate Reduction Diatabs™, URE = Urease Diatabs™, GLU = Gamma-Glutamyl Aminopeptidase Diatabs™, NAL = Nalidixan Neo-S, (S>16 mm R<16mm), CLTN = Cephalothin Neo-S (S>16mm R<16mm), AlkP = Alkaline Phosphatase Diatabs™, CAT = catalase, $+^R$ = rapid positive, PZA = Pyrazinamidase Diatabs™, COL = Colistin.

3a) Differentiation of *C. curvus*, *C. jejuni*, *Wolinella succinogenes*, and *Helicobacter pylori* (5)

All strains OXI + and MOT +.

	IAC	**CAT**	**URE**	**NO_3**
Campylobacter curvus	+ wk	0	0	+
Campylobacter jejuni	+	+	0	+
Wolinella succinogens	0	0	0	+
Helicobacter pylori	0	+	$+^R$	0^+

CAT = catalase, URE = Urease Diatabs™, NO_3 = Nitrate Reduction Diatabs™, OXI = Oxidase Diatabs™, MOT = motility, $+^R$ = rapidly positive.

3b) Campylobacter homirins, C. concisus, C. showae, B. ureolyticus

	IAC	URE	NO_3	CAT	OXI	Flag	Metro
Campylobacter homirins	0	0	V	0	+	0	S
Campylobacter concisus	0	0	+	0	V	+	S
C. showae	+	0	+	+	V	+	I/R
B. ureolyticus	0	+	+	0	+	+	S

Flag=Flagels, Metro=Metronidazole

4) Differentiation of emerging *Campylobacter* spp., Arcobacter and Helicobacter from stools

Membrane filtration onto antibiotic-free media and incubation in an H_2-enriched microaerobic atmosphere at 37 °C is a simple and costeffective protocol for the isolation of all known Campylobacter, Arcobacter and Helicobacter spp. (6 Lastovica A.J. et al.).

	IAC	PZA	HIP	Growth McC	Rapid H_2S	NAL	CLTN	Remarks
C. jejuni subsp. *jejuni*	+	+	+	+	+/0	S/R	R	
C. jejuni subsp. *doylei*	+		+	0	0	S	S	NO_3 0
C. coli	+	+	0	+	0	S	R	
C. upsaliensis	+	+	0	0	0	S	S	
Arcobacter butzleri	+	0	0	+	0	V	R	Aerotolerant COL S
Arcobacter skirrowi	+	0	0	0	0	V	V	Aerotolerant COL S
*C. fetus *)*	0	0	0	+	0	R	S^R	
C. lari	0	+	0	0	+	R	R	
C. fennelliae	+	0	0	0	0	S	S	NO_3 0, COL R
H. cinaedi	0	·	0	0	0	S	V	NO_3 +, COL R
C. hyointestinalis	0	0	0	+	0	R	V	COL S, Metro S
C. concisus	0^+	+	0	0	0	V	S	COL S, Metro S
C. sputorum	0	V	0	+	+	R	S	COL S
C. insulaenigrae	0		0	0	0	R	R	NO_3+, 42°C+
Anaerobiospirillum succinoproducns	+		0		0	R	S	

*)	CLTN	42 °C	NAL	CLTN
C. fetus subsp. *fetus*	S	+	R	S
C. fetus subsp. *venerealis*	R	0	V	S

HIP = Hippurate Hydrolysis Diatabs™, Growth McC = Growth in McConkey, NAL = Nalidixan Neo-S, CLTN = Cephalothin Neo-S, Col = colistin. NO_3 = Nitrate Reduction Diatabs™

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Indoxyl Acetate (Indoxyl acetate)	*Campylobacter jejuni* ATCC 33291	*E. faecalis* ATCC 51299

References

1) Mills C.K., Gherna R.L.: Hydrolysis of Indoxyl Acetate by Campylobacter Species. J. Clin. Microbiol. **25**, 1560-1561, 1987.

2) Sorlin P. et al: Recurrent Flexispira rappini bacteriemia in an adult patient undergoing hemodialysis: case report. J. Clin. Microbiol. **37**, 1319-23, 1999.

3) Weir S. et al: Recurrent bacteremia caused by a "Flexispira like" organism in a patient with X-linked agammaglobulinemia. J. Clin. Microbiol. **37**, 2439-45, 1999.

4) Weir S. et al: Un uncommon Helicobacter isolate from blood: evidence of a group of Helicobacter spp. pathogenic in AIDS patients. J. Clin. Microbiol. **37**, 2729-33, 1999.

5) Wetsch N.M. et al: Campylobacter curvus-associated hepatic abscesses: a case report. J. Clin. Microbiol. **44**, 1909-11, 2006.

6) Lastovica A.J. et al: Emerging campylobacter spp.: the tip of the iceberg. Clin. Microbiology Newsletter **28**, 49-55, 2006.

LYSINE DECARBOXYLASE (LDC) ORNITHINE DECARBOXYLASE (ODC)

REF No. 56811

REF No. 57011

The diagnostic tablets are based on a modified conventional enzyme test between the active ingredient and a colour indicator. The active ingredient in Lysine Decaboxylase Diatabs™ is lysine and in Ornithine Decarboxylase Diatabs™ ornithine.

Decarboxylation of lysine by lysine decarboxylase yields cadaverine, while decarboxylation of ornithine yields putrescine. The production of these amines elevates the pH of the medium, changing the colour of the indicator from yellow to blue/violet (positive). If the organism does not produce the appropriate enzyme, the suspension remains acidic (yellow).

Procedure

Prepare a dense suspension (at least McFarland No. 4) of the strain to be tested in 0.25 ml saline in a tube. Add one diagnostic tablet and **3 drops of paraffin oil** and close the tube. The oil overlayer provides anaerobic conditions necessary to avoid false positive reactions. Incubate at 35-37 °C for **4 hours** or **up to 24 hours**.

Reading of the test

After 4 hours incubation:

Positive reaction:	**Blue/violet**
Negative reaction:	Yellow, green

After 18-24 hours incubation:

Positive reaction:	**Strong violet**
Negative reaction:	Yellow, green, grey or light blue

Results

1) Both tests are well-known tests in the identification of Enterobacteriaceae and Vibrionaceae.

2) Ornithine Decarboxylase is used together with Indole and Urease in biotyping of *Haemophilus* spp. (see document **3.15.2**).

3) Ornithine Decarboxylase is used for identification of *Staphylococcus lugdunensis*/st.pseudolugdunensis

	ODC	PYR	DEFRX	Remarks
Staphylococcus lugdunensis	+	+	R(≤14mm)	Maltose +
S. epidermidis/hominis	0^{+}	0	S(≥16mm)	
Other CNS	0	V	R	
Staph. pseudolugdunensis	+	+	R	Maltose 0

4) Coryneform bacteria

	LDC	ODC
Actinomyces neuii	70	+
Dermobacter hominis	+	+
Other fermentative coryneforms	0	0

ODC = Ornithine Decarboxylase Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, DEFRX = Deferoxamine Diatabs™, LDC = Lysine Decarboxylase Diatabs™

5) *Burkholderia cepacia* complex (PYR 0, TRYP 0) (5)

See under TRYPSIN (document **3.3.5**)

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Lysine Decarboxylase (LDC) (L-Lysine)	*K. pneumoniae* ATCC 13883	*Enterobacter cloacae* ATCC 13047
Ornithine Decarboxylase (ODC) (L-Ornithine HCl)	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 13883

References

1) Schnitzler N. et al: Staph. lugdunensis: report of a case of peritonitis and easy to perform screening strategy. J. Clin. Microbiol. **36**, 812-3, 1998.

2) Kahlmeter G. et al: S. lugdunensis orsakar inte bara endokardit, 1998.

3) Leung M.J. et al: Colony variation in Staph. lugdunensis. J. Clin. Microbiol. **36**, 3096-8, 1998.

4) Früh M. et al: Use of second-line biochemical and susceptibility tests for the differential identification of coryneform bacteria. Clin. Microbiol. Infect. **4**, 332-8, 1998.

5) Henry D.A.: Phenotypic methods for determining genomovar status of the Burkholderia cepacia complex. J. Clin. Microbiol. **39**, 1073-8, 2001.

METRONIDAZOLE 5 µg (MTR.5)

REF No. 59711

Susceptibility to Metronidazole 5 µg can be used as a simple method **to screen for anaerobic bacteria**.

Procedure

The Metronidazole 5 µg diagnostic tablet (9 mm) is placed on an inoculated primary agar plate. The plate is incubated at 35-37 °C in anaerobic atmosphere for **24-48 hours**.

Apply one Metronidazole 5 µg tablet on the primary inoculum, using enriched blood agar. The tablet must be placed on the edge of the plate, otherwise growth of extremely susceptible organisms (fusobacteria) may be suppressed completely. Primary plates should be examined after incubation for 48 h and 5 days. Cell as well as colony morphology and smell are useful in the identification process of gram positive anaerobic cocci.

Reading of the test

	MTR.5
Anaerobic bacteria:	S (zone of inhibition ≥ 15 mm)
Microaerophilic bacteria	R (no zone of inhibition)
Aerobes:	R (no zone of inhibition)

Results

Gram positive **anaerobic** cocci (peptostreptococci) must be distinguished from **microaerophilic** organisms (streptococci, gemella, *Staph. saccharolyticus*).

1) Peptostreptococci (MTR.5 susceptible) and similar (most current clinical isolates) Vanco S, Col R

	GLU	α-GLU	IND	PRO	PYR	Alk P	SPS	Remarks
P. anaerobius	+	+	0	+	0	0	S	(≥ 12 mm)
Peptoniphilus. asaccharolyticus	0	0	+⁰	0	0	+	R	
Parvimonas micra	0	0	0	+⁰	+	+	R/V	
F. magna	0	0	0	0	+	V	R	
P. stomatis	+	+	0	0	0	0	S	(≥ 15 mm)
Anaerococcus vaginalis	+	V	0	0	0	V	R	LAP +, ADH+
Peptoniphilus harei	0	0	0	0	0	0	R	

GLU = Glucose Diatabs™,α-GLU = Alpha-Glucosidase Diatabs™, IND = Indole Diatabs™, PRO Proline Aminopeptidase
Diatabs™ , PYR = Pyrrolidonyl Aminopeptidase Diatabs™, Alkaline Phosphatase Diatabs™, SPS = SPS Diatabs™

2) Propionibacterium and Eubacterium spp

	MTR5
Propioribacterium spp.	zone<15 mm (I/R)
Eubacterium spp.	zone>16 mm (S)

3) Propinibacterium in human infections (Metronidazole I/R)

	Aerotolerance	**CAT**	**IND**	**NO_3**	**ESC**
P. acnes	+	+	+	+	0
P. avidum	+	+	0	0	+
P. granulosum	+	+	0	0	0
P. propionicum	0	0	0	+	0

4) Actinobacteria human (Eubacterium like) No aerotolerance. Gram positive rods.

	GLU	**CAT**	**IND**	**NO_3**	**ESC**	**ADH**	**ONPG**	**β-GLU**	**NAG**
Collinsella aerofaciens	+	0	0	0	V	V	V	V	0
Collinsella intestinalis	+	·	·	·	·	·	0	0	+
Collinsella stercoris	+	·	·	·	·	·	+	Wk	+
Eggerthella hongkongensis	0	+	0	0	·	+	0	+	0
Eggerthella lenta	0	+	0	+	0	+	0	0	0
Eggerthella sinensis	0	+	0	0	·	+	0	0	0

Quality Control

Diatabs™ (Active ingredients)	**Sensitive**	**Resistant**
Metronidazole 5 µg	*B. fragilis* ATCC 25285	*E. coli* ATCC 25922

References

1) Murdoch D.A.: Gram-positive anaerobic cocci. Clin. Microbiol. Reviews **11**, 81-120, 1998.

2) Yuli Song et al: Development of a flow chart for identification of gram-positive anaerobic cocci in the clinical laboratory. J. Clin. Microbiol. **45**, 512-516, 2007.

METRONIDAZOLE 50 µg (MTR50)

REF No. 43611

Susceptibility to Metronidazole 50 µg and S.P.S. can be used as simple means to separate four major groups of vaginal bacteria that may be confused morphologically with *Gardnerella vaginalis*.

Procedure

Use the agar diffusion method with an inoculum equivalent to McFarland 0.5 on Mueller-Hinton II agar + 5% blood. Incubate in an atmosphere with 10% CO_2.

Results

1) *Gardnerella vaginalis and Atopobium vaginae*

CAT 0, OXI 0

	MTR50	SPS	αGLU	ß-GLU	HIP	Remarks
Gardnerella vaginalis	S/R (≥12 mm S)	S (≥10 mm)	+	0	+	PRO +
G. vaginalis like organisms	R	R	·	·	$+^0$	
Lactobacilli	R	R	+	·	90	
Coryneforms	R	R	+	·	V	
Bifidobacteria	S	R	$+^0$	·	0	
Atopobium vaginae	R	R	0	0	·	PRO +, ADH +, LAP +, Vanco S

MTR50 = Metronidazole 50 µg Diatabs™, SPS = SPS Diatabs™, -GLU = Alpha-Glucosidase Diatabs™, ß-GLU = Beta- Glucosidase Diatabs™, HIP = Hipurate Hydrolysis Diatabs™

Resistance of *G. vaginalis* to metronidazole may have arisen from widespread use of this drug to treat bacterial vaginosis (3). Resistance to metronidazole is now common among *G. vaginalis* isolates.

Quality Control

Diatabs™ (Active ingredients)	**Sensitive**	**Resistant**
Metronidazole 50 µg	*G. vaginalis* ATCC 14018	*E. coli* ATCC 25922

References

1) Piot P.: Gardnerella, Streptobacillus, Spirillum, and Calymmatobacterium. pp. 483-487 in Manual of Clinical Microbiology, 5th ed., Balows A. et al (eds), ASM, 1991.

2) Bastida Vilá M.T.: Gardnerella vaginalis bacteremia in adult male. J. Clin. Microbio. Infect. Dis. **16**, 400- 1, 1997.

3) McLean N.W. et al: Growth inhibition of metronidazole-susceptible and metronidazole-resistant strains of Gardnerella vaginalis by lactobacilli in vitro. Appl. Environm. Microbiol. **62**, 1089-2, 1996.

NITRATE REDUCTION (NO_3)

REF No. 43711

Contain sodium molybdate and potassium nitrate.

Procedure 1

Prepare a dense "milky" suspension (at least McFarland No. 4) from the strain to be tested in 0.25 ml saline in a tube. Add one Nitrate Reduction tablet and close the tube. Incubate at 35-37 °C for **4 hours** or **18-24 hours**. After incubation **add 1 drop of N,N-Dimethyl-α-Naphthylamine sol.** and **1 drop Sulfanilic acid sol.** Read within 2 minutes.

Reading of the test

Positive reaction: **Red/pink**

Negative reaction: Colourless, light pink

Results

1) Most aerobes give a positive reaction. The following give a negative reaction:

	NO_3
Acinetobacter	0
Moraxella	0
Flavobacterium	0
some *Pseudomonas* spp.	0

2) Cocco-bacillary *Neisseria* spp. / Moraxella / Psychrobacter / Pasteurella (Oxidase +)

	CAT	GLU	NO_3	TRIB	COL	Pigment	Remarks
N. elongata subsp. *glycolytica*	0	$+^0$	0	0	S	+	
N. elongata subsp. *elongata*	+	0	0	0	S	+	
N. elongata subsp. *nitroreducens*	0	0	+	0	S	+	
N. weaveri	+	0	0	0	S	0	
N. bacilliformis (4)	0^+	0	$+^0$	$+^0$	S	+ wk	
Kingella denitrificans	0	+	+	0	R	0	
Kingella kingae	0	+	0	0	R	0	
Kingella potus	0	+	0	+	R	+	

Moraxella catarrhalis	+	0	$+^0$	+	V	0	
Psychrobacter spp.	+	+	V	V	·	0	URE +
Pasteurella spp.	+	+	+	·	S	0	IND $+^0$, ODC$+^0$, URE 0^+

CAT = catalase, GLU = Glucose Diatabs™, NO_3 = Nitrate Reduction Diatabs™, TRIB = Tributyrin Diatabs™, COL = Colistin 10 µg Diatabs™ (S ≥ 12 mm, R < 10 mm).

Procedure 2

When testing **anaerobes**, the tablet can also be placed on an inoculated plate, which is incubated for **24-48 hours**. After incubation **1 drop each of N,N-Dimethyl--Naphthylamine sol.** and **Sulfanilic acid sol.** is added to the tablet.

Reading of the test

A **pink or red** colour is interpreted as **positive** indicating reduction of nitrate to nitrite.

Results

1) Among anaerobes the following give a positive reaction:

	NO_3
Bacteroides ureolyticus group	+
Veillonella	+
Propionibacterium acnes	+
Some *Clostridia* spp.	+
Eubacterium lentum	+
Bilophila wadsworthia	+
Wolinella/Campylobacter	+

2) Differentiation of Propionibacteria (Metro I/R, Col R, Kana S, Vanco S)

	NO_3	**IND**	**ß-XYL**	**CAT**
Propionibacteria acnes	$+^0$	+	0	+
Propionibacteria avidum	0	0	+	+
Propionibacteria granulosum	0	0	0	+
P. propionicum (*Arachnia*)	+	0	0	0

NO_3 = Nitrate Reduction Diatabs™, IND = Indole Diatabs™, ß-XYL=Beta-Xylosidase Diatabs™ and CAT = catalase.

Quality Control

Diatabs™	Positive	Negative
(Active ingredients)		
Nitrate Reduction (Sodium Molybdate 40 µg, Potassium nitrate)	E. coli ATCC 25922	S. saprophyticus ATCC 15305

References

1) Wideman P.A., Citronbaum D.M., Sutter V.L.: Simple disk technique for detection of nitrate reduction by anaerobic bacteria. J. Clin. Microbiol. **5**, 315-319, 1977.

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3) Funke G. et al: Clinical Microbiology of Coryneform bacteria. Clin. Microbiol. Reviews **10**, 125-159, 1997.

4) Lundgren B. et al: Two cases of endocarditis caused by Neisseria elongata subsp. nitroreducens and phenotypic differentiation from Kingella denitrificans. J. Clin. Microbiol. and Infect. **4**, 514-8, 1998.

NOVOBIOCIN 5 µg (NOVO5) Neo-Sensitabs™

REF No. 76312

May be used in the diagnostic work to differentiate **the *Staphylococcus saprophyticus* group** (causing urinary tract infections in young women) from **other coagulase negative staphylococci**. The *S. saprophyticus* group is resistant to Novobiocin 5 µg Neo-Sensitabs™, while other staphylococci are sensitive. Use Mueller-Hinton II agar.

In anaerobe bacteriology Novobiocin 5 µg Neo-Sensitabs™ may be used as a presumptive test to differentiate ***Peptostreptococcus anaerobius/indolicus*** that are sensitive: (MIC <1.6 µg/ml), from other peptostreptocci that are resistant to novobiocin (MIC >25 µg/ml), i.e. zone size below 13 mm.

Procedure

For anaerobes use FAA + 5% blood or supplemented Brucella Blood agar with an inoculum equivalent to McFarland 0.5. Use current susceptibility testing media for staphylococci/ pediococci.

Results

1a) *Staphylococcus saprohyticus* group

	McFarland 0.5 (Kirby- Bauer)	**Semi-confluent growth**
	Resistant (zone)	
S. saprophyticus, *Staphylococcus xylosus,* *Staphylococcus cohnii,* *S. cohnii* subsp. *urealyticum,* *Staphylococcus sciuri,* *Staphylococcus lentus*	< 13 mm	< 15 mm
	Sensitive (zone)	
Other staphylococci	≥ 14 mm	≥ 16 mm

1b) *Staphylococcus scuiri* group (Novo R, OXI+)

	TRIB	CEL	MAL	RAF	SUC	AlkP
S. scuiri	0	+	V	0	+	+
S. lentus	+	+	+[0]	+	+	+[0]
S. fleurettii	Vwk	0	+	0	+	0
S. vitulinus	45	v	0	0	+	0

2) *Staphylococcus hominis/epidermidis*

	NOVO5	DEFRX	FOSFO	MSE
S. hominis subs. *hominis*	S	S	R (<28 mm)	9
S. hominis subs. *novobiosepticus*	R	S	R (<28 mm)	9
S. epidermidis	S[R]	S	S (>30 mm)	90
Other CNS	V	R	V	V

NOVO5 = Novobiocin 5 µg Neo-S, DEFRX = Deferoxamine Diatabs™ (S 16mm, R 14mm),
FOSFO = Fosfomycin Neo-S, MSE = Mannose Diatabs™

3) Anaerobes

	Sensitive (zone)
Peptostreptococcus anaerobius, P. indolicus, *P. heliotrinreducens,*	≥ 14 mm
	Resistant (zone)
P. asaccharolytica, *F .magna, M. micros,* *A. prevotii, A. tetradius*	< 13 mm

4) Pediococci (Vanco R, BE+, PYR 0, LAP+)

	NOVO5	MAL
Pediococcus acidilactici	S	0
Pediococcus pentosaceus	R	+

NOVO5 = Novobiocin 5 µg Neo-S and MAL = Maltose Diatabs™

References

1) Wren M.W.D., Eldon C.P., Dakin G.H.: Novobiocin and the differentiation of peptococci and peptostreptococci. J. Clin. Path. **30**, 620-622, 1977.

2) Casals J.B., Pringler N.: Identification of staphylococci using a combination of chromogenic substrates and sensitivities towards Furazolidone, Novobiocin and Colistin. Workshop on Pathogenesis of Wound and Biomaterial-Associated Infections, Lund University, 1989.

3) Wegener H.C.: Diagnostic value of phage typing, antibiogram typing, and plasmid profiling of Staph. hyicus from piglets with exudative epidermitis J.Vet.Med. 13-20, 1993.

4) Devriese L.A.: A simple identification scheme for coagulase negative staphylococci from bovine mastitis. Research in Vet. Science **57**, 240-4, 1994.

5) Weinstein M.P. et al: Clinical importance of identifying CNS isolated from blood cultures: evaluation of Microscan panels versus a conventional Reference Method. J. Clin. Microbiol. **36**, 2089-92, 1998.

O/129 (Vibriostaticum) (O/129)

REF No. 45411

Vibrios are sensitive to the vibriostatic agent O/129 (2,4-diamino 6,7 di-isopropyl pteridine). The diffusible amount is 150 µg per tablet. The O/129 is useful for differentiation of **Vibrios** from **Enterobacteriaceae** and **Aeromonas**. O/129 is also useful in the differentiation of corynebacteria.

Procedure

A plate of Oxoid Blood Agar Base (CM271) containing 0.5% NaCl is seeded with the culture under test and the O/129 150 µg diagnostic tablet is applied. The plates are incubated for **24 hours** before reading sensitivity.

If commercial sensitivity agar is used instead of CM271, many of the marine vibrio strains will not grow, but in addition many enterobacteria will show a degree of sensitivity to O/129.

Strains with acquired resistance against trimethoprim will also be resistant to O/129.

Reading of the test

Sensitive: ≥16 mm

Resistant: <16 mm

Results

1a) Differentiation of *Aeromonas* spp.

	ODC	LDC	ADH	ARA	TRYP	Remarks
Aeromonas hydrophilia	0	+	+	+	27	
Aeromonas caviae	0	0	+	+	+	
Aeromonas veronii (*sobria*)	0	+	+	0	+⁰	
Aeromonas (veronii)	+	+	0	0	0	URE +

1b) Vibrio/Aeromonas/Plesiomonas/Photobacterium

Most strains are: OXI +, GLU +

	O/129	ADH	ODC	MAN
Vibrio spp.	S	0^{+}	$+^{0}$	+
Plesiomonas shigelloides	S	+	+	0
Aeromonas	R	$+^{0}$	0^{+}	+
Photobacterium spp.	S	+	0	0

Note:

Strains showing resistance to trimethoprim or trhimethoprim + sulfa connot reliably be tested with O/129.

ODC = Ornithine Decarboxylase Diatabs™, LDC = Lysine Decarboxylase Diatabs™, ADH = Arginine Dihydrolase Diatabs™, ARA = Arabinose Diatabs™, TRYP = Trypsin Diatabs™, MAN = Mannitol Diatabs™

1c) Vibrio/Enterobacteriaceae/Pasteurellaceae

	MOT	OXI	Alk P	O/129	High salt
Vibrio spp.	+	+	V	S	Growth
Enterobacteriaceae	+	0	0	R	V
Pasteurellaceae	0	+	+	S	No growth

MOT = motility, OXI = Oxidase Diatabs™, Alk P = Alkaline Phosphatase Diatabs™, High salt = medium with high salt content.

2) Corynebacteria nonlipophilic, fermentative

	O/129	Growth20°C	NAG	LAP	MAL	AMP	α-GLU	AlkP	Colonies
Corynebacterium striatum	S		0	82	0	S	0	+	res
C. minutissimum	S		89	+	+	S	0	+	
C. amycolatum (F-2)	R		0	0	80	R^{S}	0^{+}	+	Dry multires.
Corynebacterium xerosis	S	0	0	$+^{0}$	+	R^{S}	$+^{0}$	+	yellowish
Corynebacterium hansenii	S	·	0	+	+	S	0	0	yellowish
Corynebacterium freneyi	S	+	0	+	+	S	+	+	wrinkled
Corynebacterium riegelii	S	0	·	+	+	S	·	V	White, URE $+^{R}$, GLU 0

3) Corynebacteria

	O/129	PZA
Corynebacterium diphtheriae	S	0
Corynebacterium imitans	R	wk
Corynebacterium striatum	S	+

O/129 = O/129 150 µg Diatabs™, NAG = Beta-N-Acetylglucosaminidase Diatabs™, LAP = Leucine Aminopeptidase Diatabs™, MAL = Maltose Diatabs™, PZA = Pyrazinamidase Diatabs™, AMP = Ampicillin 33 µg Neo-S, ADH = Arginine Dihydrolase Diatabs™, OCT = Ornithine Decarboxylase Diatabs™, α-GLU = Alpha-Glucosidase Diatabs™,Colonies. AlkP=Alkaline Phorplatase D.T, res=resistant to several antibiotics.

Quality Control

Diatabs™ (Active ingredients)	**Sensitive**	**Resistant**
O/129 150 µg (2,4-Diamino-6,7-diisopropylpteridine phosphate salt)	*Kocuria rhizophila* ATCC 9341	*E. coli* ATCC 25922

References

1) Lee J.V.: Identification of Aeromonas, Vibrio and related organisms, pp. 152-165 in Identification methods for microbiologists. Skinner F.A., Lovelock D.W. (Ed.s.), Acad. Press London, N.Y. 1979.

2) Baumann P., Schubert H.W.: Vibrionaceae, page 535 in Bergey's Ma nual of Systematic Bacteriology, vol. 1, 1984.

3) Dalgaard P.: Qualitative and quantitative characterization of spoilage bacteria from packed fish. Intl. J. Food Microbiol. **26**, 319-333, 1995.

4) Abbott S.L. et al: Misidentification of unusual Aeromonas spp. as members of the genus Vibrio: a continuing problem. J. Clin. Microbiol. **36**, 1103-4, 1998.

5) Früh M. et al: Use of second-line biochemical and susceptibility tests for the differential identification of coryneform bacteria. Clin. Microbio. Infect. **4**, 332-8, 1998.

6) Renaud F.N.R.: Differentiation of Corynebacterium amycolatum, C. minutissimum and C. striatum by carbon substrate assimilation tests. J. Clin. Microbiol. **36**, 3698-3702, 1998.

7) Abbot S.L. et al: The genus Aeromonas: biochemical characteristics, atypical reactions and phenotypic identification schemes. J. Clin. Microbiol. **41**, 2348-57, 2003.

8) Renaud F. N. R. et al: Corynebacterium hansenii sp nov an α-glucosidase negative bacterium related to C. xerosis. Int. I. System Evol. Microbiol. **57**, 113-16, 2007.

OPTOCHIN (OPT) OXGALL (OXG)

REF No. 44211

REF No. 44311

Optochin is an agent capable of inhibiting growth of pneumococci, but not alpha-streptococci or other streptococci. Optochin Diatabs™ contain 10 µg of diffusible optochin and are useful for the presumptive identification of **pneumococci**.

Oxgall is useful being a substitute of the bile solubility test; each tablet contains 1000 µg diffusible oxgall. Oxgall should always be tested on TSA agar or TSA +5 % blood.

Procedure

Pneumococci (incubated in an atmosphere containing CO_2 on an agar with blood) show an inhibition zone ≥18 mm around Optochin diagnostic tablets, while streptococci show inhibition zones of < 16 mm. In the event of inhibition zones of 16-17 mm, the test is repeated with optimum inoculum (McFarland 0.5).

Pneumococci incubated aerobically show a zone of inhibition ≥20 mm, but the preferred method is CO_2 atmosphere (6). The optimal blood agar is TSA with 5 % sheep blood. With Oxgall, pneumococci show a zone of ≥ 19 mm.

Nunes et al (9) reported optochin resistance amone pneumococci colonizing healthy children in Portugal. Bile solubility test was positive.

Reading of the test

	Optochin	**Oxgall**
CO_2 atmosphere:		
Pneumococci:	≥18 mm	≥ 19 mm
Streptococci :	< 16 mm	≤ 17 mm
Aerobe atmosphere:		
Pneumococci:	≥20 mm	
Streptococci:	< 18 mm	

Results

1) Differentiation of non-beta-haemolytic streptococci

	OPT	BE	PYR	HIP	OXG	α-GAL	ADH	Remarks
S. pneumoniae	S	0	0	0	S	+	+	TRE +
Group B strep (non ß-haem)	R	0	0	+	R	·	·	
S. bovis	R	+	0	·	R	·	·	
Viridans streptococci	R	0^{+}	0^{+}	0^{+}	R	·	·	
NVS (*Abiotrophia* spp.)	R	0	+	V	·	·	·	
S. mitis	R/S	0	0	0	R	·	0	TRE 0
S. pseudopneumoniae	R/S	0	0	0	R	0	+	TET R^{S}, ERY R^{S}, TRE V

OPT = Optochin Diatabs™, BE = Bile Esculin Diatabs™, PYR = Pyrrolidonyl Aminopeptidase Diatabs™, HIP = Hippurate Hydrolysis Diatabs™, NVS = Nutritionally variant streptococci, OXG = Oxgall Diatabs™ (S ≥ 19 mm, R ≤ 17 mm), α- GAL = Alpha-Galactosidase Diatabs™, ADH = Arginine Dihydrolase Diatabs™,.

2) Differentiation of *S. pneumoniae, S. pseudopneumoniae* and *S. mitis/oralis* group (7,8)

	OPT	ADH	1% Deox	0.1% Deox
S. pneumoniae	S	+	S	S
S. pseudopneumoniae	R^{S}	+	R	S
S. mitis/oralis group	R^{S}	0^{+}	R	S

OPT = Optochin Diatabs™, ADH = Arginine Dihydrolase Diatabs™, 1 % Deox = 1 % sodium deoxycholate lysis, 0.1 % Deox = 0.1 % sodium deoxycholate lysis.

3) Non-motile, slow growing anaerobic/microaerophilic gram negative rods, nitrate positive (Kana S, Vanco R, Col S, NO_3+, often Metro R)

	OXI	CAT	OXGALL	URE	MOT	LAP	IAC	Remarks
Bact ureolyticus	+	0^+	S	+	0	+	·	
Campylobacter showae	+	+	S	0	$+^0$	·	+	
Campylobacter concisus	+	0	S	0	$+^0$	·	0	
Campylobacter rectus	$+^0$	0^+	S	0	+	+	+	Rifa S
Campylobacter gracilis	0	0^+	S	0	0	+	0^+	Rifa R
Sutterella wadsworthiensis	0	0	R	0	0	+	·	
Bilophila wadsworthia	0	+	R	$+^0$	0	·	·	Alk P+
Eikenella corrodens	+	0	S	0	0	·	·	LDC +, ODC +

4) Non-motile, slow growing, anaerobic gram negative cocci (Vanco R, Kana S, Col S, Oxgall S, Metro S)

	OXI	CAT	PYR	GLU	LAP	NO_3	Very small cells
Acidaminococcus intestini	0	0	+	0	+	0	0
Acidaminococcus fermentans	0	0	0	0	·	0	0
Veillonella spp	0	0	V	0	·	+	+ Col S
Megasphera elsdenii	·	·		+		0	0
Dialister spp	0	0	0	0	+	0	+ Col R

GLU=Glucose D.T (add 3 drops of paraffin oil)

Quality Control

Diatabs™ (Active ingredients)	**Sensitive**	**Resistant**
Optochin 10 µg (Ethylhydrocuprein HCl)	*S. pneumoniae* ATCC 49619	*S. bovis* ATCC 15351
Oxgall 1000 µg (Oxgall)	*S. aureus* ATCC 25923	*B. fragilis* ATCC 25285

References

1) Ragsdaler R.A., Sanford J.P.: Interfering effect of incubation in carbon dioxide on the identification of pneumococci by optochin discs. Appl. Microbiol. **22**, 854-855, 1971.

2) Gardam M.A.: Optochin revisited: defining the optimal type of blood agar for presumptive identification of Strept. pneumoniae. J. Clin. Microbiol. **36**, 833-4, 1998.

3) Ruoff K.L.: Streptococci. Manual Clinical Microbiology 6th Ed., 299-307, 1995.

4) Kellog J.A. et al: Identification of Strept. pneumoniae revisited. J. Clin. Microbiol. **39**, 3373-5, 2001.

5) Christensen A. et al: Pneumococci and bile solubility. Clin. Microbiol. Infect. **6**, Suppl. 1, 163, 2000.

6) Arbique J.C. et al: Accuracy of phenotypic and genotypic testing for identification of S. pneumoniae and description of S. pseudopneumoniae sp. nov. JCM **42**, 4686-96, 2004.

7) Keith E.R. et al: Characteristics of S. pseudopneumoniae isolated from purulent sputum samples. J. Clin. Microbiol. **44**, 923-7, 2006.

8) Balsalobre at al: Molecular characterization of disease-associated streptococci of the mitis group that are optochin susceptible. J. Clin. Microbiol. **44**, 4163-71, 2006.

9) Nunes S. et al: Optochin resistance among S. pneumoniae strains, colonizing healthy children in Portugal. J. Clin. Microbiol. **46**. 321-4, 2008.

OXIDASE (OXI)

REF No. 45711

The oxidase test is useful in the presumptive identification of Neisseria as well as for miscellaneous gram-negative bacteria (Non-Fermenters, Vibrionaceae, Campylobacter, etc.).

Oxidase Diatabs™ contain the substrate NNN'N'-tetramethyl-p-phenylenediamine 2 HCl, which is very sensitive.

Procedure

Lay a thick filter paper in an empty petri dish and place an Oxidase diagnostic tablet on it. Add one drop of **saline on top of the tablet**, wait 60 seconds and add **another drop of saline on top of the tablet**.

When the filter paper around the tablet is wet**, the colony is immediately smeared** onto the wet filter paper approx. 3-8 mm apart from the edge of the tablet using a plastic or platinum loop (Nichrome and iron containing wires give false positive reactions).

Reading of the test

Make the reading **within 2 min.** of smearing the filter paper. The colony turns **blue/purple** when the strain is **oxidase positive**. Use a positive control in cases of weak positive reactions.

Results

Among the oxidase <u>positive</u> microorganisms are:

Neisseria	Aeromonas	Pasteurella
Vibrio	most *Pseudomonas* spp.	Flavobacterium
Alcaligenes	Moraxella	Campylobacter
Plesiomonas.		

Among the oxidase <u>negative</u> are:

Staphylococci	streptococci	anaerobes
Enterobacteriaceae	Acinetobacter	*Stenot. maltophilia*
Haemophilus.		

Differentiation of Anaerobispirillum from Campylobacter:

	OXI	Ery	CAT
Campylobacter spp.	+⁰	S (≥ 30 mm)	+⁰
Anaerobispirillum spp. (succiniproducens)	0	R (≤ 23 mm)	0

OXI = Oxidase Diatabs™, Ery = Erythromycin Neo-S, CAT = catalase.

Quality Control

Diatabs™ (Active ingredients)	**Positive**	**Negative**
Oxidase (Tetramethyl-p-phenylenediamine)	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922

References

1) Gadberry J.L., Clemmons K., Drumm K.: Evaluation of methods to detect oxidase activity in the genus Pasteurella. J. Clin. Microbiol. **12**, 220- 225, 1980.

2) Tee W. et al: Three cases of Anaerobispirillum succiniproducens bacteremia confirmed by 16 S + RNA Gene sequencing. J. Clin. Microbiol. **36**, 1209-13, 1998.

Reagents

Reagents are used together with some of the Diatabs™. An overview of these Diatabs™ is given in the table. Ninhydrin Solvent, Aminopeptidase and Kovacs' Reagents are available from Rosco. The other reagents are easily prepared. Follow the safety guidelines for the chemicals being used. For quality control use the reagent together with the recommended Diatabs™ when testing positive and negative QC strains.

Reagent	**REF No.**	**Use with Diatabs™ (REF No.)**
Aminopeptidase Reagent	92231	46711, 46811, 46911, 47011, 47211
Kovacs' Reagent	92031	57611, 58411, 59111, 59011, 57611 (IND)
Ninhydrin Solvent	91731	56711 (HIP)
N,N-Dimethyl-α-Naphtylamine		43711 (NO_3)
Sulfanilic acid solution		43711 (NO_3)
Ferric Chloride 10 % solution		57911, 57811 (TDA)
Alpha-naphthol solution		57711 (VP)
40 % KOH		57711 (VP)
Ferrous ammonium sulphate solution		59811 (PZA)

N,N-Dimethyl-α-Naphthylamine:

Dissolve 600 mg N,N-Dimethyl-α-Naphthylamine (Sigma D 4011 or Fluka 40860) in 30 ml Acetic acid 100 % and dilute to 100 ml with water, purified. Store in the refrigerator in a brown glass bottle away from light.

Sulfanilic acid solution:

Dissolve 800 mg Sulfanilic acid i 30 ml Acetic acid 100% and dilute to 100 ml with water, purified. Store in the refrigerator in a brown glass bottle away from light.

Ferric Chloride 10 % solution:

Dissolve 10 g ferric chloride $FeCl_3 \cdot 6\ H_2O$ in water, purified to make 100 ml.

Alpha-naphthol solution:

Dissolve 5 g α-naphthol in 100 ml of absolute ethanol. Store in the refrigerator in a brown glass bottle away from light.

40 % KOH:

Dissolve 40 g of potassium hydroxide in 100 ml of carbon dioxide free water, purified.

Ferrous ammonium sulphate solution 5%:

Dissolve 5 g of ferrous ammonium sulfate in 100 ml of purified water. Use only freshly prepared or stored at –20 °C.

Useful TABLES for bacterial identification / differentiation

Document / Section no.

1) ENTEROBACTERIACEAE

Differentiation of *Citrobacter* spp.....3.15.2

Differentiation of Proteus / Morganella / Providencia from others 3 15.5;3.37.0

Differentiation of Enterobacter 3.5.0

Differentiation of *Enterobacter (Cronobacter) sakazakii* from other *Enterobacter* spp. 3.20.6

Differentiation of Cronobacter spp from Enterobacter spp 3.20.6

Enterobacter (Cronobacter) sakazakii and similars.....3.20.6

Differentiation of *Yersinia* spp. 3 36.0;3.42.0

Differentiation of *Yersinia enterocolitica* pathogenic serotype 3.10.0;3 34.0;3.36.0
Salmonella and Shigella serotypes..... 3.3.1

2) NON-FERMENTERS

Differentiation of most important non-fermenters..... 3.3.5

Differentiation of most common resistant non-fermenters..... 3.3.4

Identification of *Pseudomonas aeruginosa* (C-390, Ps.aeruginosa Screen) 3 11.0;3.33.0

Differentiation of *Ps. fluorescens, Ps. putida* and *Ps. stutzeri*..... 3.3.5

Differentiation of *Acinetobacter baumanii / lwoffi* *3 3.1;3.20.9*

Differentiation of *Burkholderia cepacia* complex..... 3.3.5

Differentiation of *Ralstonia / Cupriavidus* spp. 3.14.0

Differentiation of *Shewanella alga / Shewanella putrefaciens* 3.31.0

Differentiation of gram negatives from cystic fibrosis patients 3.33.0

Differentiation between *Burkholderia*, *Ralstonia* and *Pandoraea* spp. 3.3.4

Differentiation of *Burkholderia cepacia* complex, *B. gladioli*, *R. picketti* and *R. manitolilytica*.*3.3.5*
Differentiation of Chryseobacterium/Elizabethkingia spp..... *3.3.5*

Screening tests for Burkholderia pseudomallei 3.14.0

Doc. no.

3) VIBRIO / AEROMONAS / PLESIOMONAS

Differentiation of Vibrio, Aeromonas, Plesiomonas, Photobacterium..... 3.28.0

Differentiation of *Aeromonas* spp. 3.28.0

Differentiation of the most common *Vibrio* spp. (human)..... 3.15.2

Differentiation of Vibrio/Enterobacteriaceae/Pasteurellaceae 3.28.0

4) STAPHYLOCOCCI / MICROCOCCI / KITOCOCCUS

Identification of most important staphylococci 3.2.0

Identification of most common human staphylococci 3.3.4

Differentiation of coagulase negative staphylococci (human) 3 14.0;3.18.0
Differentiation of coagulase negative mastitis staphylococci 3 14.0;3.18.0
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Alphabetic INDEX of Abbreviations and Codes

A) ACM = Acetamide Hydrolysis Diatabs™ (55711)

ADH = Arginine Dihydrolase Diatabs™ (56211)

ADO = Adonitol Diatabs™ (52011)

AER = Aerotolerant strains

ALA (dALA) = Porphyrin (d-Ala) Diatabs™ (57311)

Alk P = Alkaline Phosphatase Diatabs™ (55911)

ARA = l-Arabinose Diatabs™ (52111)

ANAgr = Anaerobic growth

AMP (AMP33) = Ampicillin 33 µg Neo-Sensitabs™ (70412)

AmpC = AmpC beta-lactamases

ARG=Arginine Aminopeptidase Diatabs™ (10611)

B) BACIT = Bacitracin 40 U Neo-Sensitabs™ (70812)

BaciLow (BaL) = Bacitracin Low 0.4U Diatabs™ (40211)

BE = Bile Esculin Diatabs™ (40411)

BrG = Brilliant green 100 µg Diatabs™ (40511)

BOR (BORON) = Phenylboronic Acid 250 µg Diatabs™ (10411)

β-haem=Beta haemolysis

C) C-390 = C-390 40 µg Diatabs™ (41611)

CAT = Catalase

CEL = Cellobiose Diatabs™ (Non-stock)

CCFA = CCFA medium (Clostridium difficile)

CIT = Citrate Diatabs™ (56511)

CL500 = Cloxacillin 500 µg Diatabs™ (10311)

CLTN (CLOTN) = Cephalothin 66 µg Neo-Sensitabs™ (72912)

or Cephalothin 30 µg Neo-S (60612)

COL (Co.10) = Colistin 10 µg Neo-Sensitabs™

Col dry adh = Colonies dry adherent CYC = Cycloheximide Diatabs™ (58911)

D) DEF (DEFRX) = Deferoxamine 250 µg Diatabs™ (59611)
DUL = Dulcitol Diatabs™ (Non-stock)

E) ESBL = Extended spectrum betal-lactamases

ESC = Esculin Hydrolysis Diatabs™ (56611)

F) Fosfo (FOSFO) = Fosfomycin (Fosfomycin+Glucose-6-Phosphat) Neo-Sensitabs™ (74212)

FRU = Fructose Diatabs™ (Non-stock)

α-FUC = Alpha-Fucosidase Diatabs™ (50111)

ß-FUC = Beta-Fucosidase Diatabs™ (59911)

Fura (FURAZ) = Furazolidone 50 µg Neo-Sensitabs™ (74412)

Flag=flagels

G) GAL = Galactose Diatabs™ (Non-stock)

α-GAL = Alpha-Galactosidase Diatabs™ (50211)

Gel (GEL) = Gelatine hydrolysis

Genta 250 (GN250) = Gentamicin 250 µg Neo-Sensitabs™ (43012)

GLU = Glucose Diatabs™ (52611)

α-GLU = Alpha-Glucosidase Diatabs™ (50411)

ß-GLU = Beta-Glucosidase Diatabs™ (50511)

γ-GLU = Gamma-Glutamyl Aminopeptidase Diatabs™ (46711)

H) HCF = Human clumping factor

HIP = Hippurate Hydrolysis Diatabs™ (56711)

HLR = High Level Resistance

I) IMP (IMIPM) = Imipenem 15 µg Neo-Sensitabs™ (74612) or Imipenem 10 µg Neo-S (61212)

IAC = Indoxyl Acetate Diatabs™ (59551)

IND (IN) = Indole Diatabs™ (Non-stock)

INO = Inositol Diatabs™ (Non-stock)

INU = Inulin Diatabs™ (52711)

K) Kana 500 (KA500) = Kanamycin 500 µg Neo-Sensitabs™ (43112)

L) LAC = Lactose Diatabs™ (52811)

LAP = Leucine Aminopeptidase Diatabs™ (46811)

LDC = Lysine Decarboxylase (LDC) Diatabs™ (56811)

LEC = Lecithinase

LIP = Lipase LIPO=Lipophilic

LDC/IND = LDC/Indole (Lysine decarboxylase/Indole) Diatabs™ (58411)

M) MAL = Maltose Diatabs™ (52911) MALON = Malonate

MAN = Mannitol Diatabs™ (53011)

α-MAN = Alpha Mannosidase Diatabs™ (50711)

MBL = Metallo-beta-lactamases

McConk. = Growth in McConkey Agar

MGP = Methyl-α-D-glucopyranoside

MEL = Melibiose Diatabs™ (53211)

MTR50 = Metronidazole 50 µg Diatabs™ (43611)

MTR.5 = Metronidazole 5 µg Diatabs™ (59711)

MOT = Motility

MR = Methyl Red

MRS = Man, Sharp, Rogosa broth. MSE = Mannose Diatabs™ (53111)

MTM = Growth on modified Thayer-Martin medium

Mupi (MUPIR) = Mupirocin 10 µg Neo-Sensitabs™ (75712)

N) NA35 = Growth on nutrient agar at 35 °C

NAG (ß-NAG) = Beta-N-Acetylglucosaminidase Diatabs™ (50011)

NAL (NALID) = Nalidixan 130 µg Neo-Sensitabs™ (75812) or Nalidixic acid Neo-S

NO_3 = Nitrate Reduction Diatabs™ (43711)

Novo (Novo-5) (NOVO5) = Novobiocin 5 µg Neo-Sensitabs™ (76312)

NVS =Nutritionally variant streptococci

O) O/129 = O/129 (Vibriostaticum) 150 µg Diatabs™ (45411)

ODC = Ornithine Decarboxylase (ODC) Diatabs™ (57011)

ODC/IND = ODC/Indole Diatabs™ (59111)

ONPG = ONPG (Beta-Galactosidase) Diatabs™ (50311)

OPT = Optochin 10 µg Diatabs™ (44211)

OXG = Oxgall 1000 µg Diatabs™ (44311)

OXI = Oxidase Diatabs™ (45711)

P) PGUA (PGA) = Beta-Glucuronidase Diatabs™ (50611)

PGUA/IND = PGUA/Indole (Beta-Glucuronidase/Indole) Diatabs™ (59011)

PIGM = Pigment production

Poly (CO150) = Polymyxins 150 µg Neo-Sensitabs™ (77512)

PRO = Proline Aminopeptidase Diatabs™ (46911)

PSAER (PsS) = Ps. aeruginosa Screen 80 µg Diatabs™ (59311)

PYR = Pyrrolidonyl Aminopeptidase (PYR) Diatabs™ (47011)

PYR (1h)= PYR rapid test

PZA = Pyrazinamidase Diatabs™ (59811)

R) R = Resistant

R^S = Most strains resistant

RAF = Raffinose Diatabs™ (53311)

RHAM = Rhamnose Diatabs™ (Non-stock)

Res=multiresistant

RIB = Ribose Diatabs™ (Non-stock)

RIFA (Rifa) (RIFAM) = Rifampicin 30 µg Neo-Sensitabs™ (77712)

RM = Rapid motility (4 hours at 35 °C)

S) S = Sensitive (susceptible) S^R = Most strains sensitive

SAL = Salicin Diatabs™ (Non-stock)

SFT = Sugar fermentation tests

SOR (SORB) = Sorbitol Diatabs™ (53711)

SPS = S.P.S. 1000 µg Diatabs™ (44611)

SUC = Sucrose Diatabs™ (53811)

SUP = Superoxol (30 % H_2O_2)

Strep 500 (ST500) = Streptomycin 500 µg Neo-Sensitabs™ (44712)

T) TDA or IND = TDA or Indole (Tryptophan Deaminase or Indole) Diatabs™ (57811)

TEL = Tellur 500 µg Diatabs™ (45011)

TRE = Trehalose Diatabs™ (53911)

TRIB = Tributyrin Diatabs™ (48811)

TRYP = Trypsin Diatabs™ (47211)

TTR = Tetrathionate Reductase Diatabs™ (57411)

U) URE (UR) = Urease Diatabs™ (57511)

URE/IND = Urease/Indole Diatabs™ (57611)

URE/TDA = Urease/TDA (Urease/Tryptophan Deaminase) Diatabs™ (57911)

V) V = Variable

Vanco (Van.5) = Vancomycin 5 µg Neo-Sensitabs™ (79312)

VP = Voges-Proskauer Diatabs™ (57711)

wk = weak

X) XYL = Xylose Diatabs™ (54011)

ß-XYL = Beta-Xylosidase Diatabs™ (50811)

$+^{R}$	=	rapidly positive
+	=	More than 90 % strains positive
$+^{0}$	=	75 - 90 % strains positive
V	=	26 - 74 % strains positive
0^{+}	=	10 - 25 % strains positive
0	=	Less than 10 % strains positive

If a number is written in the table, it refers to the percentage of positive strains.