DIATABS™

User's Guide

Diagnostic Tablets
for Bacterial Identification

Latest version

DIATABS™- DIAGNOSTIC TABLETS for BACTERIAL IDENTIFICATION

The **DIATABS™**, Diagnostic Tablets (D.T.), developed by Rosco 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 8th Ed. 2009 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.dk and updated information is continuously included.

ROSCO Diagnostica A/S is welcoming any feedback and questions on bacterial identification from users directly (info@rosco.dk) or through our representatives.

ROSCO

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

AMINOPEPTIDASES:

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

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SUGAR FERMENTATION TESTS:

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	Cellobiose (25)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
	Dulcitol (25)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
	Fructose (25)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
	Galactose (25)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
52611	Glucose (50)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
	Inositol (25)	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 (25)	Enterobacteriaceae/Staphylococci etc.	**3.36.0**
53621	Salicin (25)	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**
54021	d-Xylose (25)	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, in order 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.

3.1.0 ACETAMIDE HYDROLYSIS (ACM)

REF No. 55721

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 Acetmide 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.

3.2.0 ALKALINE PHOSPHATASE (Alk P)

REF No. 55921

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: Colorless 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 D.T., PYR (1h) = Pyrrolidonyl Aminopeptidase D.T. (1h incubation), ODC = Ornithine Decarboxylase D.T., DEFRX = Deferoxamine D.T., URE = Urease D.T., Poly = Polymyxins 150 µg Neo-S, MAL = Maltose D.T., SUC = Sucrose D.T., SOR = Sorbitol D.T., HCF = Human clumping factor, NO_3 = Nitrate Reduction D.T., VP = Voges Proskauer D.T., BaciLow = Bacitracin Low D.T. ($S \geq 10$ 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.

3.3.0 AMINOPEPTIDASES ARGININE AMINOPEPTIDASE (ARG)

AMINOPEPTDASES

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 color in case of positive reactions.

Range

The actual range of aminopeptidases (substrates) comprises:

Arginine Aminopeptidase	(ARG)	(10061)
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 color 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 specific 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. 10061

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 color.

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 color 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*

3.3.1 GAMMA-GLUTAMYL AMINOPEPTIDASE (γ-GLU)

REF No. 46711

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

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 Gamma-glutamyl 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 color 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) Neisseria/Moraxella (HTM + (V), OXI +, CAT +^{0})

	γ-GLU	NA35	SUP	TRIB	Remarks
N. menigitidis	+	0^+	V	0	
N. gonorrhoeae	0	0	+	0	Co 10 R
N. lactamica	0	$+^0$	V	0	ONPG +
N. cinerea	0	+	0	0	Co 10 S
N. polysaccharea	0	+	0	0	Co 10 R
M. catarrhalis	0	+	+	+	NO_3 +
Kingella denitrificans	0	·	0	0	Co 10 R, NO_3 +, CAT 0

Most *N. gonorrhoeae* are PRO (proline aminopeptidase) positive, but approx. 5 % are negative (5).

2) Acinetobacter

	γ-GLU	β-XYL
A. baumanii/calcoaceticus	$+^0$	+
A. lwoffi	0	0

3) Helicobacter

	γ-GLU	IAC
Helicobacter pylori	+	0
Helicobacter cinaedi	0	0wk
Helicobacter fennelliae	0	+

γ-GLU = Gamma-Glutamyl Aminopeptidase D.T., NA35 = Growth in nutrient agar at 35 °C, SUP = Superoxol (30 % H_2O_2) hydrogen peroxide, TRIB = Tributyrin D.T., HTM = Growth on Modified Thayer Martin medium, OXI = Oxidase D.T., CAT = Catalase, β-XYL = Beta-Xylosidase D.T., IAC = Indoxyl Acetate D.T., Co 10 = Colistin 10 µg (S≥10 mm).

4a) Identification of clinically most common *Nocardia* spp. (3). 4 hours' tests except URE (7)

	ESC	PYR	γ-GLU	α-GLU	α-MAN	URE	45 °C	Remarks
N. abscessus *	·	0	+	+	0	+	0	Imip S/R
N. brasiliensis	·	0	+	+	+	+	0	Imip R
N. cyriacigeorgica *	+	0	+	+	0	0	+	AMC I/R, Imip S
N. farcinica	+	+	+	+	0	$+^0$	+	AMC S, Tobra R, Ery R

N. nova	0	+	0	+	0	V	0/+	Tobra I/R, AMC I/R, Ery S
N. paucivorans	0	0	+	0	0	0	+	
N.asiatica	·							
N. wallacei	+					+	+wk	
N. blacklockiae	+					+	0	

*) Members of the *N. asteroides* complex.
ESC=Esculin hydrolysis, PYR = Pyrrolidonyl Aminopeptidase D.T., γ-GLU = Gamma-Glutamyl-Aminopeptidase D.T., α-GLU = Alpha-Glucosidase D.T., α-MAN = Alpha-Mannosidase D.T., URE = Urease D.T., 45 °C = growth at 45 °C.

4b) Identification of *Nocardia* spp. by antibiogram (6, 7)

	CIPRO	AMOX	AMC	CEFTR	IMIP	CLARI	TOBRA
N. abscessus	R	S	S	S	V	V	S
N. brasiliensis	R	R^S	S	V	R	R	S
*N. cyriacigeorgica**	R	V	I/R	S	S	R	S,AMIKA**S**
N. farcinica	S	R	S	R	S	R	R
N. nova	·	S	I/R	V	S	S	V
N.asiatica	R	·	·	S	S	I	S, T+S**R**
N. wallacei	·	·	S	S/I	R	R	R
N. blacklockiae	·	·	S^R	S^R	·	R	R

AMOX = Amoxycillin Neo-S, AMC = Amoxycillin+Clavulanate Neo-S, CEFTR = Ceftriaxone Neo-S, IMIP = Imipenem Neo-S, CLARI = Clarithromycin Neo-S, Tobra = Tobramycin Neo-S, Ery = Erythromycin Neo-S, CIPRO= Ciprofloxacin Neo-Sensitabs.
*) Schlaberg et al (7) found synergism between Imipenem (10 µg disk) and Amikacin (30 µg disk) separated by 35 mm. with N. cyriacigeorgica.

5) Salmonella and Shigella (4) serotypes

	γ-GLU	Remarks
S. typhimurium	80 %	
S. enteritidis	+	
S. blegdam	+	
S. berta	+	
S. gallinarum/pullorum	+	
S. typhi	+	
S. arizonae	0	PGUA 0
S. diarizonae	+	PGUA +
S. dublin	0	
S. naestved	0	
S. kiel	0	
Shigella dysenteriae		
- serotypes 3 to 9	+	
- serotypes 1, 2 and 10	0	
Shigella sonnei	0	
Salmonella serotype III a	0	
Salmonella serotype III b	+	

γ-GLU = Gamma-Glutamyl-Aminopeptidase D.T.

6) Differentiation of species and sub-species of Salmonella

	ONPG (2h)	γ-GLU	PGUA	SORB	Galacturonate
S. enterica I	0	+	V	+	0
S. salamae II	0	+	V	+	+
S.arizonae IIIa	+	0	0	+	0
S. diarizonae IIIb	+	+	+	+	+
S. hoUtenae IV	0	+	0	+	+
S. indica VI	V	+	V	0	+
S. bongori V	+	+	0	+	+

PGUA: Beta glucuronidase DT, SORB= Sorbitol DT, ONPG=ONPG DT (2 hours incubation), γ-GLU = Gamma-Glutamyl-Aminopeptidase D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Gamma-Glutamyl Aminopeptidase (Gamma-Glutamyl-β-Naphthylamide)	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922

References (γ-GLU)

1) Mc Nulty C.A.M et al: Rapid identification of *Campylobacter Pylori* by preformed enzymes. J. Clin. Microbiol. **25**, 1683-6, 1987.
2) Nebreda T. et al: Urethritis caused by *Neiss. meningitidis* serogroup C. Clin. Microbiol. Infect. **5**, 57-8, 1999.
3) Wauters G et al: Distribution of Nocardia species in clinical samples and their routine rapid identification in the laboratory. J. Clin. Microbiol. **43**, 2624-8, 2005.
4) Giammanco G. et al: Interet taxonomique de la recherche de la γ-glutamyltransferase chez les Enterobacteriaceae. Ann Microbiol. (Inst. Pasteur) **131A**, 181-7, 1980.
5) Blackmore T. et al: Characterization of prolyl-iminopeptidase-deficient N. gonorrhoeae. J. Clin Microbiol., **43**, 4189-90, 2005.
6) Glupczynski Y. et al: Determination of antimicrobial susceptibility patterns of Nocardia spp. for clinical specimens by E-test. Clin Microbiol. Infect. 12, 905-912, 2006.
7) Schlaberg R, et al: Nocardia cyricigeorgica an emerging pathogen in the USA. J. Clin. Microbiol. **46**, 265-273, 2008.

3.3.2 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 color.

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 color 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 D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., Van5 = Vancomycin 5 µg Neo-S (S ≥15 mm, R ≤13 mm), ADH = Arginine Dihydrolase D.T., BE = Bile Esculin D.T., PGUA = Beta-Glucuronidase D.T.

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 nonlipophilic fermentative (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 multidrugresistant
Res= resistance to ≥ 5 drugs

4) Corynebacteria nonlipophilic nonfermentative

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 D.T., LAP = Leucine Aminopeptidase D.T., NO_3 = Nitrate Reduction D.T., MAL = Maltose D.T., O/129=O/129 150 µg D.T. (S ≥16 mm, R < 16 mm), NAG = Beta-N-acetylglucosominidase D.T., DNase, URE = Urease D.T., 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 D.T., LAP = Leucine Aminopeptidase D.T., Inulin D.T.

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 D.T., LAP = Leucine Aminopeptidase D.T., VP = Voges-Proskauer D.T., MAL = Maltose D.T., SUC = Sucrose D.T.

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.

3.3.3 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*

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

	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	.	

PRO = Proline Aminopeptidase D.T., CCFA growth = Growth on CCFA medium, ONPG = ONPG Beta-Galactosidase D.T., Alk P = Alkaline Phosphatase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T.,
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 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 D.T., ESC = Esculin Hydrolysis D.T., 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 D.T., URE = Urease D.T., 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 D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., α-GLU = Alpha-Glucosidase D.T., IND = Indole D.T., SPS = SPS D.T., GLU = Glucose D.T. (add 3 drops paraffin oil), Alk P = Alkaline Phosphatase D.T.

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 D.T., NAG = Beta-N-Acetylglucosaminidase D.T., α-GLU(2h) = Alpha-Glucosidase D.T. (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.
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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.

3.3.4. 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 color 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 color 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, staphylococcci, 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 color 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 color)
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. lugdunensis	+	+	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 D.T., ODC = Ornithine Decarboxylase D.T., VP = Voges-Proskauer D.T., Poly = Polymyxins 150 µg Neo-Sensitabs (S ≥ 14 mm, R ≤ 12 mm), Alk P = Alkaline Phospotase D.T., DEFRX = Deferoxamine D.T. (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 D.T., α-MAN = Alpha-Mannosidase D.T., VP(24h) = Voges-Proskauer D.T. (incubation 24 h), TRIB = Tributyrin D.T. and XYL = Xylose D.T., BE = Bile Esculin D.T., ADH = Arginine Dihydrolase D.T., 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 D.T., LDC = Lysine Decarboxylase D.T., ARA = Arabinose D.T., URE = Urease D.T., TTR = Tetrathionate Reductase D.T.

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 D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., ACM = Acetamide Hydrolysis D.T., TTR = Tetrathionate Reductase D.T., ADH = Arginine Dihydrolase D.T., COL = Colistin 10 µg D.T. (S ≥ 12 mm), PSAER = Ps. aerigunosa Screen D.T. (R ≤ 14 mm), NO_3 = Nitrate Reduction D.T., ß-XYL = Beta-Xylosidase D.T., α-Man = Alpha-Mannosidase D.T., IMIP = Imipenem Neo-S, LDC = Lysine Decarboxylase D.T., DEFRX = Deferoxamine D.T. (S ≥ 16 mm, R ≤ 14 mm).

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

	NO3	OXI	DEF	PYR	LDC	42°C	ONPG	URE	Alk P	Remarks
Burkholderia cepacia complex	V	+	R^S	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 D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., LDC = Lysine Decarboxylase D.T., URE = Urease D.T., Alk P = Alkaline Phosphatase D.T., Merop = Meropenem Neo-S, Genta = Gentamicin Neo-S, Tobra = Tobramycin Neo-S, LAP = Leucine Aminopeptidase D.T., 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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6) Devriese L.A. et al: Identification of Enterococcus species isolated from foods of animal origin. Intl. J. Food Microbiol. 26, 187-197, 1995.
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9) Sasaki T. et al: Reclassification of phenotypically identified Staph. intermedius strains. J. Clin. Microbiol. 45, 2770-78, 2007.

3.3.5 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 color. 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 color 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 D.T. 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 (S)
Sh. putrefaciens	0	100	30	+	0	R	100	
Sh. algae	0	+	·	+	0	R	R	
Elisab. meningoseptica	0	100	+	0	0	R	R	αMAN+,IND $+^0$
Sphing. paucimobilis	0	100	+	0	0	R	19	IND 0, ß-XYL +, URE 0, Pigm +
Sphing. multivorum	0	100	+	0	0	R	R	IND 0, URE +, αMAN+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 S, URE 0, 0/129 S
Inquilinus limosus	·	100	V	·	0	·	R	IND 0, PRO +, NAG + ß-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 +
Burkh. gladioli	·	·	·	·	·	R	R	ONPG +, OXI 0, Pigm $+^0$
Cupriavidus pauculus (IVc-2)		+			0	R	S	NO_3 0 , URE$+^R$
D) OXI +, PYR 0, TRYP +								
Ps. fluorescens (PYR 62)	0^+	0	0	0^+	+	R	100	
Sphing. paucimobilis	0	100	+	0	0	R	19	β-XYL+
Brev. diminuta	0	100	0	0	0	92	R	NO_3 0, γGLU+
Brev. vesicularis	0	100	+	0	0	100	R	NO_3 0, γGLU 0
Ps. stutzeri	0 wk	0	$+^0$	V	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	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	γGLU+, PRO+, URE+, NO3+
Olig. ureolytica	0	0	0	V	0^+	·	100	γGLU +, URE$+^R$, NO_3 +
Olig. urethralis	0	0	0	0	0	100	100	γGLU +, URE 0, NO_3 0
Pandoraea spp.	0	+	0	0	V	·	R	Merop R, LDC 0, ONPG 0, LAP +, CAT +, MOT +

TRYP = Trypsin D.T., α-FUC = Alpha-Fucosidase D.T., β-XYL = Beta-Xylosidase D.T., NAG = Beta-N-Acetylglusosaminidase D.T., NO_3 = Nitrate Reduction D.T., IND = Indole D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., TRIB = Tributyrin D.T., α-MAN = Alpha-Mannosidase D.T., LDC = Lysine Decarboxylase D.T., IMP = Imipenem Neo-S (R = no zone), ACM = Acetamide Hydrolysis D.T., Alk P = Alkaline Phosphatase D.T., α-GLU = Alpha-Glucosidase D.T., TTR = Tetrationate Reductase D.T., ADH = Arginine Dihydrolase D.T., DEF = Deferoxamine D.T. (S ≥ 16 mm, R ≤ 14 mm), COL = Colistin 10 µg D.T. (S ≥ 13 mm, R ≤ 10 mm), PSAER = Ps. aeruginosa Screen D.T. (S ≥ 16 mm, R ≤ 14 mm), MAN = Mannitol D.T., NO_3 = Nitrate Reduction D.T., PRO = Proline Aminopeptidase D.T., VP = Voges Proskauer D.T., TTR = Tetrathionate Reductase D.T. , OXI = Oxidase D.T., CAT = catalase, SUC = Sucrose D.T., MOT = motility, URE = Urease D.T., Merop = Meropenem Neo-S, ONPG = ONPG Beta-Galactosidase D.T., LAP = Leucine Aminopeptidase D.T.
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
B. pyrrocinia (IX)			0	$+^0$	+	+	0	V	+	0	+	$+^0$	XYL +, MAL +, LACT + 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 D.T., LDC = Lysine Decarboxylase D.T., ODC = Ornithine Decarboxylase D.T., 42 °C = growth at 42 °C, PIGM = Pigment production (brown or yellow), SUC = Sucrose D.T., MAL = Maltose D.T., OXI = Oxidase D.T., XYL = Xylose D.T., LACT = Lactose D.T., PRO = Proline Aminopeptidase D.T., NAG = Beta-N-Acetylglucosaminidase D.T., COL = Colistin 10 µg Neo-S ($S \geq 13$ mm, $R \leq 10$ mm) DEFRX = Deferoxamine D.T. ($S \geq 16$ mm, $R \leq 14$ mm). GLU = Glucose D.T., ADH = Arginine Dihydrolase D.T., Alk P = Alkaline Phosphatase D.T., LAP = Leucine Aminopeptidase D.T., CAT = catalase, MOT = motility, Merop = Meropenem Neo-S, CIT = Citrate D.P., TRIB = Tributyrin D.T.

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 D.T., α-GLU = Alpha-Glucosidase D.T., 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 D.T., OXI = Oxidase D.T., ONPG = ONPG D.T., DEF = Deferoxamine D.T., COL = Colistin D.T.

6) Differentation of Chryseobacterium/Elizabethkingia spp.

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

	PIGMred	*McConkey*	*ONPG*	*URE*	*IND*	*ESC*		
Chryseob. hominis	0	0		0	0		+	
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) Differenation of most common periodontal pathogens

	TRYP	*αFUC*	*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)

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3.4.0 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 D.T. (bile) (44311), Brilliant Green D.T. (40511) and the antibiotic tablets: Vancomycin 5µg Neo-Sensitabs (45111), Kanamycin 500 µg Neo-Sensitabs (43111), Colistin 10 µg D.T. (41811), and Rifampicin 30 µg Neo-Sensitabs (26112) are placed on a plate of FAA + 5% blood or supplemented Brucella Blood Agar, which has been inoculated with an inoculum corrsponding 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, $\mathbf{S}^R$ = 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

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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.
5)Bernard D. et al: Bilophila wadsworthia bacteremia in a patient with gangrenous appendicitis. CID, **18**, 1023-4, 1994.
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3.5.0 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 alcalinization of the medium and a change of color 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 D.T., MR = Methyl Red, α-GLU = Alpha-Glucosidase D.T., ODC = Ornithine Decarboxylase D.T.

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*.

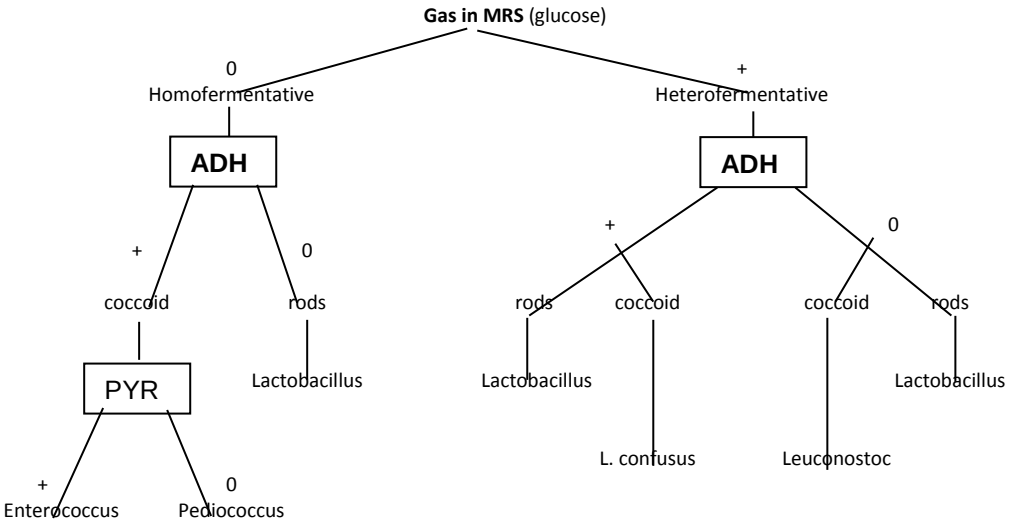
3) Non-fermenters

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 D.T., PGUA = Beta-Glucuronidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., MR = methyl red, MRS = Man, Sharp, Rogosa broth, NAG = N-Acetylglucosaminidase D.T., α-GAL = Alpha-Galactosidase D.T.

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.

3.6.0 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 D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., MUPI = Mupirocin Neo-S (S ≥ 16 mm, R < 16 mm), OPT = Optochin D.T.

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
Bacitracin low 0.4 U	*S. pyogenes* ATCC 12344	*S. bovis* ATCC 15351

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.

3.7.0 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.

3.8.0 BETA LACTAMASE (Acido)

REF No. 45521

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 color of the indicator (bromcresol purple) from violet to yellow.

Procedure

Prepare a heavy (at least McFarland No. 4) 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 color 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.

3.9.0 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	10031
Boronic Acid 250 µg	BORON	10041
Dipicolinic Acid 250 µg	D.P.A	10051

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.

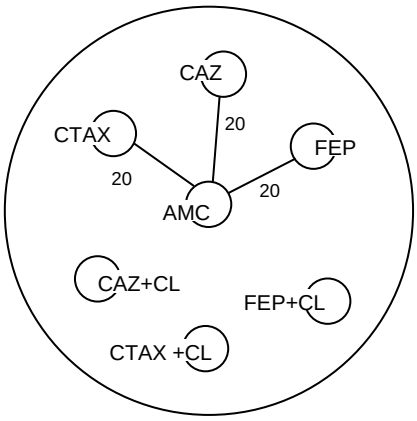


Fig 1. ESBL - Enterobacteriaceae

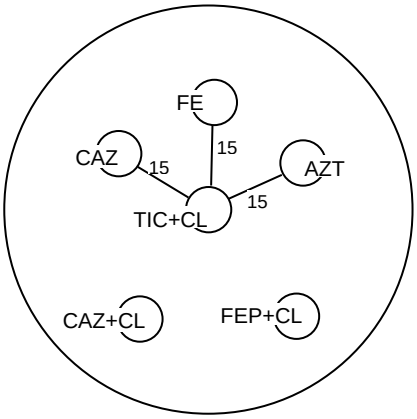


Fig 2. ESBL – Non-fermenters

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 Acinetobacters 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.dk).

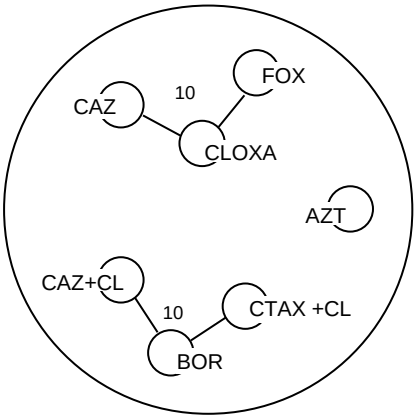


Fig 3 AmpC

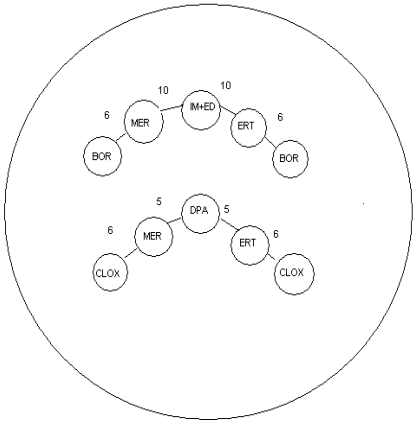


Fig 4 Screening of carbapenemases (Metallo-β-lactamases and KPC)

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.dk)

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.
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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.

3.10.0 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: Colorless/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 color 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 D.T., SAL = Salicin D.T. and PZA = Pyrazinamidase D.T. 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 D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., ADH = Arginine Dihydrolase D.T. 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 D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., SORB = Sorbitol D.T., MAN = Mannitol D.T.α-GAL = Alpha-Galactosidase D.T.

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 D.T., CAMP = CAMP reaction, URE = Urease D.T. ESC = Esculin Hydrolysis D.T., αFUC = Alpha-Fucosidase D.T., αGLU = Alpha-Glucosidase D.T., NAG = Beta-N-Acetylglucosaminidase D.T., ARA = Arabinose D.T., 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 D.T. (S ≥ 15 mm, R = no zone), PGUA = Beta-Glucuronisase D.T., PRO = Proline Aminopeptidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., 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.

3.11.0 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.

3.12.0 CITRATE (CIT)

REF No. 56511

Diagnostic Tablets for testing alcalinization 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.

3.13.0 CYCLOHEXIMIDE (CYC)

REF No. 58921

Cycloheximide (actidione) is a chemical substance which shows activity against several species of fungi. Cycloheximide Diagnostic Tablets 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 Diagnostic Tablet 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.

3.14.0 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. Particulary 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 includes: *S. aureus*, *S. hæmolyticus*, *S. warneri*, *S. simulans*, *S. capitis*, *S. lugdunensis*, *S. schleiferi*, *S. auricularis*, *S. saprophyticus*, *S. xylosus*, *S. cohnii*.
DEFRX = Deferoxamine D.T.

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 D.T., Fosfo = Fosfomycin Neo-S, Novo = Novobiocin 5 µg D.T., ODC = Ornithine Decarboxylase D.T, PYR(1h) = Pyrrolidonyl Aminopeptidase D.T. (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 D.T., Novo = Novobiocin 5 µg D.T., 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 D.T., Poly = Polymyxin Neo-S, VP(4h) = Voger Proskauer D.T. (4 hours incubation), MAL = Maltose D.T., TRE = Trehalose D.T., PYR (1h) = Pyrrolidonyl Aminopeptidase D.T. (1 h incubation).

5) Ralstonia/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 D.T., (S ≥16 mm), MAN = Mannitol D.T., Alk P = Alkaline Phosphatase D.T., URE = Urease D.T., $+^{R}$ = rapid positive, CAT = catalase, OXI = Oxidase, PYR = Pyrrolidonyl Aminopeptidase D.T., TRYP = Trypsin D.T.

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. B. cepacia show 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.

3.15.0 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:

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.

3.15.1 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 over layer 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 **color 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.

3.15.2 ODC / INDOLE (ODC/IND)

REF No. non-stock (59121)

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 over layer 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 D.T., DUL = Dulcitol D.T., ESC = Esculin Hydrolysis D.T., MALON = Malonate, MEL = Melibiose D.T., RAF = Raffinose D.T., ADON = Adonitol D.T., -XYL = Beta-Xylosidase D.T.

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 D.T., URE = Urease D.T.

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

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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3.15.3 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: Colorless

Indole

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

3.15.4 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 **color 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 D.T. SUC = Sucrose D.T., Factor X D.T., Factor V D.T.

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	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	0	
Gallibacterium anatis	+	0	0	0	+	V	$+^0$	+	V	α-GLU +
Avibacterium avium	$+^0$	0	0	0	0	0	+	0	0	
Avibacterium gallinarum	+	0	0	0	0	+	+	0	0^+	
Avibacterium paragallinarum	0	0	0	0	0	V	0	+	+	

CAT = catalase, URE/IND = Urease/Indole D.T., ODC = Ornithine Decarboxylase D.T., MAL = Maltose D.T., TRE = Trehalose D.T., MAN = Mannitol D.T., SOR = Sorbitol D.T., DUL = Dulcitol D.T., 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 D.T., NO_3 = Nitrate Reduction D.T., URE/IND = Urease/Indole D.T.

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

3.15.5 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 color 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.)

3.16.0 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. Streptomtycin 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.

3.17.0 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.

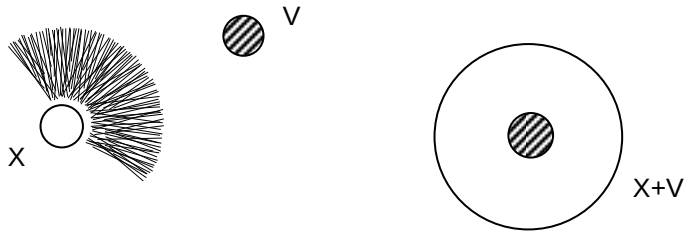
Procedure

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).

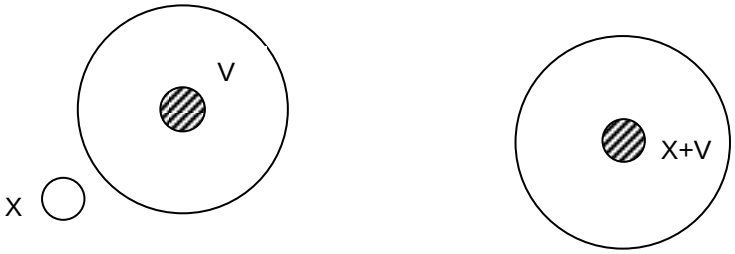
Fig. 1



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.
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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.

3.18.0 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 D.T., 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.

3.19.0 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**	**Mupi**	**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 D.T. (incub. 2 h at 37 °C, McF 3) (9), α-GAL = Alpha-Galactosidase D.T., OXA = Oxacillin Neo-S, ADH = Arginine Dihydrolase D.T., ARA = l-Arabinose, MAN = Mannitol D.T., SUC = Sucrose D.T., TEL = Tellur 500 µg D.T., ß-MAN = Beta-mannosidase, NAG = N-acetyl glucosaminidase D.T., VP = Voges-Proskauer D.T.,

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 D.T., 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 D.T.

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 D.T., PRO = Proline Aminopeptidase D.T., PGUA = Beta-Glucuronidase D.T., H_2S = Hydrogen sulphide production. Haem = Haemolysis.

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3.20.0 GLYCOSIDASES

General description

The chromogenic glycosidases tests are based upon enzymatic release of yellow-colored 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)	(50021)
Alpha-Fucosidase	(α-FUC)	(50111)
Beta-Fucosidae	(ß-FUC)	(59921)
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:	Colorless

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.

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3.20.1 BETA-N-ACETYLGLUCOSAMINIDASE (ß-NAG)

REF No. 50021

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^+).

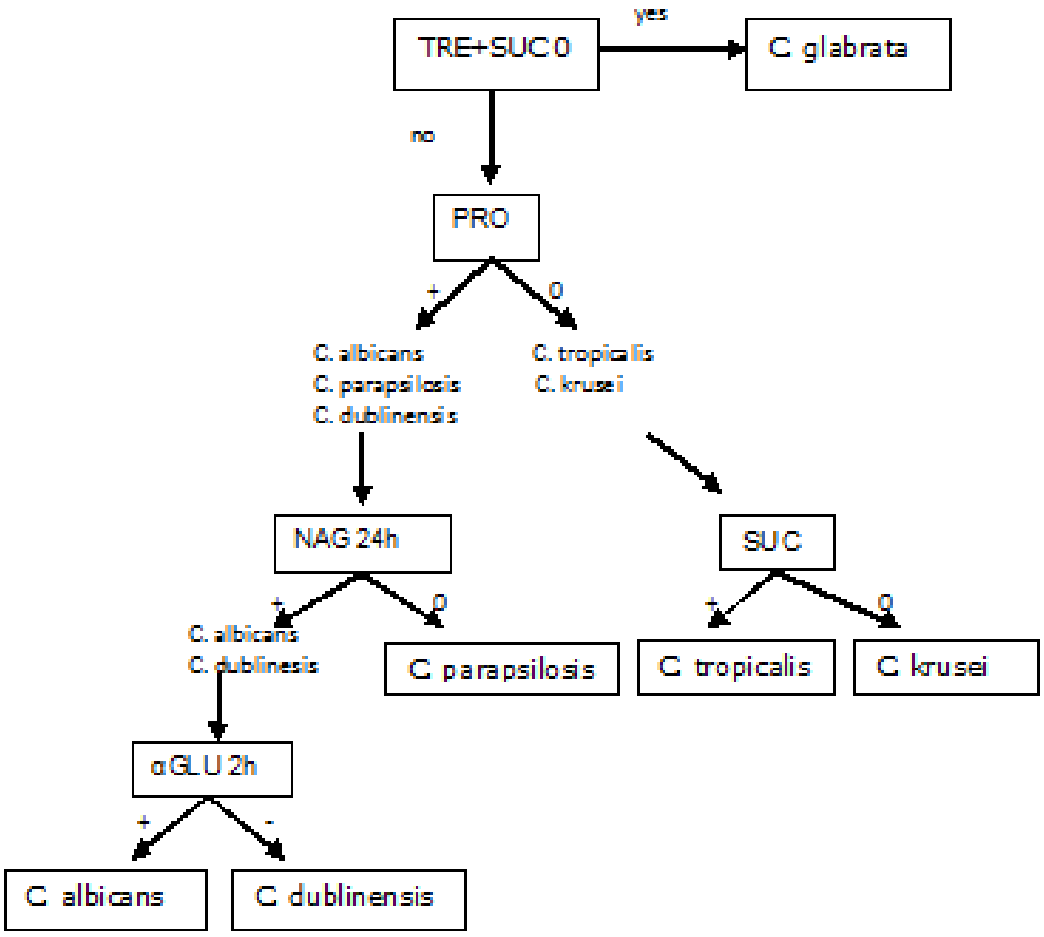
NAG = Beta-N-Acetylglucomaninidase D.T., ONPG = ONPG D.T., PZA = Pyrazinamidase D.T., PRO = Proline Amino-peptidase D.T., 42 °C = Growth at 42 °C in Sabouraud Glucose Agar, XYL = d-Xylose D.T.

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 D.T., SUC = Sucrose D.T., CYC = Cycloheximide D.T. (S ≥ 25 mm, R < 25 mm). AlkP = Alkaline Phosphatase D.T., 42 °C = Growth at 42 °C in Sabouraud Glucose Agar, 2h αGLU = Alpha-Glucosidase D.T. (incubation 2 hours). NAG is read after 24 hours' incubation (if negative, after 4 hours)

Algoritm



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 D.T.

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.
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3.20.2 ALPHA- FUCOSIDASE (α-FUC)

REF No. 50111

Results

1) Streptococci

	α-FUC
S. gordonii	+
S. sanguinis	0

2) Anaerobe gram negative rods (Oxgall S, Brilliant Green S, Col R, Vanco 5 R, Kan R, Metro S, Red fluorescence, CAT 0) non-pigmented saccharolytic

	ARA	ARG	α-FUC	αGAL	ESC	NAG	ß-XYL	Remarks
Prev. maculosa	+		0	+	+	+	·	SUC+ ,Gel 0
Prev. histicola	0		+	+	0	+	·	SUC +, Gel +
Prev. manceiensis	0		+	+	+	+	·	
Prevotella bergensis	+		0	0	+		+	IND 0, SUC 0, RAF 0, CEL+Gel0
Prevotella AIP-261-03	·		+	+	0		·	SUC +
Prev. multisaccharivorax	·		0	+	+		·	
Prec. amnii	·		0	+	+	+	·	
Prevotella disiens	0	0	0	0	0	0	0	α-GLU +
Prevotella oralis	0	+	+	+	+	+	0	
Prevotella bivia	0	+	+	0/+	0	+	0	Col R, CEL 0, RAF 0
Prevotella buccae	+	0	0	·	+	0	+	SUC+, Gel+
Prevotella buccalis	0	+	+	+	+	+	0	
Prev. veroralis	0	+	+	w	+	+	0	Kana R, CAT 0, Red fluorescence
Prev. pleuritidis	0		+	0	0	+	·	LAP+, PYR+, ONPG+,αGLU+
Prevotella multiformis	0		$+^0$	+	0		·	CEL +, RAF +
Prevotella baroniae	0		+	+	+	+	·	ONPG +
Prevotella marshii	0		0	0	0	0	0	SUC 0, MAL +, GEL+ α-GLU
Prev. timonensis	0		+	0	wk		·	
Prevotella massiliensis	0		0	0	0		0	IND +, OXI + KAN S non-saccharolyt
Prev. copri	+		+	0	+	0	·	
Prev. stercorea	0		+	+	0	+	·	

3) Porphyromonas human origin (Oxgall S, BrG S, Vanco 5 S)

	α-FUC	TRYP	IND	NAG	Remarks
P. asaccarolytica	$+^{0}$	0^{+}	$+^{0}$	0	
P. gingivalis	0	+	+	+	
P. endodentalis	0	0	+	0	
P. catoniae	+	+	0	+	Pigm 0
P.(levii like) = somerae	0	0	0	+	
Tannerella forsytheansis	+	+	V	+	PGUA +, Vanco R
P. uenonis	0	·	+	0	

4) Anaerobe gram negative rods, pigmented (OXG S, BrG S, Vanco R, Kana R, Metro S, Red fluorescence, CAT0, αGLU+, TRYP 0)

	α-FUC	α-GAL	IND	ARG	CEL	LIP	NAG	Remarks
Prevotella melaninogenica	+	+	0	+	0	+	+	ESC V
P. intermedia/nigrescens	$+^{0}$	0	+	+	0	+	0	
Prevotella denticola	+	+	0	0	0	+	+	
Prevotella loescheii	+	+	0^{+}	0	+	+	+	
Prevotella corporis	0	0	0	+	0	0	0	TRYP $+^{0}$
Prevotella pallens	0	0	+	·	0	0	0	
Prev. histicola	+	+	0	·	0	·	+	LAP0, ESC 0
Prev. falsenii	+	0	+	·	0	·	+	
Prev. micans	+	+	+	·	+	·	+	ESC 0

5) *Bacteroides fragilis* group (OXG R, BrG S, Vanco R, Kana 500 R, Col R, ESC+^{0}, Pigm 0)

	α-FUC	PGUA	LAP	IND	ß-GLU	α-GAL	ARG	TRE	RHAM	ARA	CAT	Remarks
Bacteroides fragilis	+	·		0	+	+	+	0	0	0	+	
Bacteroides vulgatus	+	·	0	0	0	+	$+^{0}$	0	+	+	0^{+}	ESC 0
Parabacteroides distasonis	0	0		0	+	+	+	+	+	0	$+^{0}$	
Bacteroides cacae	95	·		0	+	+	+	+	$+^{0}$	+	0^{+}	
Parabacteroides merdae	0	+		0	0	+	+	+	+	0^{+}	0^{+}	PYR +
Parabacteroides finegoldii	0	0	0	0	0	+		0	·	+	·	ADH 0, LAP 0
Parabacteroides goldsteinii	0	+		0	+	+		+	·	0	V	ß-XYL +
Bacteroides plebeius	+	+		0	+	+		0	+	+	0	CEL +, NAG+
Bact. coprophilus	+	0	+		+	+		0	0	0	·	
Bacteroides coprocola	+	0	0	0	+	+		0	+	0	0	
Bacteroides massiliensis	+	0	+	0	+	+		0	0	0	0	
Bacteroides dorei	+	+		0	+	+		0	+	+	0	NAG + ESC 0, CEL 0
Bact. xylanisolvens	+	0	0	0	+	+		+	+	+	0	SAL+, NAG+
Bact. thetaiotaomicron	+	·	+	+	+	+	+	$+^{wk}$	+	+	+	SAL 0^{+},CEL+
Bacteroides ovatus	+	·	+	+	+	+	0	+	+	+	$+^{0}$	SAL +

Bacteroides uniformis	+	·		+	+	+	0	0^{+}	0	+	0^{+}	SAL +
Bacteroides stercoris	$+^{0}$	·		+	+	0	0	0	+	0	0	ADH +, CEL 0
Bacteroides eggerthii	0	·		+	+	0	V	0	$+^{0}$	+	0	SUC 0
Bacteroides nordii	0	·		+	+	+		0	+	0	0	ADH 0, CEL +
Bacteroides salyersae	0	·		+	+	+		0	+	+	0	SUC +
Bacteroides intestinalis	+	0		+	+	+		0	+	+	·	SAL 0, NAG+
Alistipes finegoldii (pigm+)	+	0	0	+	0^{+}	+		0		0	0	ONPG+OXG R
A. underdonkii (pigm+)	+	0	0	+	$+^{0}$	+		0		0	0wk	ONPG, + OXG R, NAG+

Note: When testing sugar fermentation (CEL, TRE, ARA, SAL) against anaerobes, add 3 drops of paraffin oil before incubation.

α-FUC = Alpha-Fucosidase D.T., TRYP = Trypsin D.T., IND = Indole D.T., αGLU = Beta-Glucosidase D.T., ESC = Esculin Hydrolysis D.T., α-GAL = Alpha-Galactosidase D.T., CEL = Cellobiose D.T., OXG = Oxgall D.T., BrG = Brilliant Green D.T., LIP = Lipase, TRE = Trehalose D.T., ARA = Arabinose D.T., CAT = Catalase, ß-XYL = Beta-Xylosidase D.T., NAG = Beta-N-Acetylglucosaminidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., PGUA = Beta-Glucuronidase D.T., SAL = Salicin D.T., COL = Colistin 10 µg D.T. (S ≥ 13 mm, R ≤ 10 mm), αGLU = Alpha-Glucosidase D.T., Oxgall D.T. (S ≥ 10 mm, R < 10 mm), BrG = Brilliant green D.T. (S ≥ 10 mm, R < 10 mm), Kana 500 = Kanamycin 500 Neo-S (S ≥ 10 mm, R < 10 mm) Col = Colistin 10 µg Neo-S (S ≥ 10 mm, R < 10 mm) Vanco = Vancomycin 5 µg Neo-S (S ≥ 20 mm, R ≤ 18 mm), ADH = Arginine Dihydrolase D.T., SUC = Sucrose D.T. , RAF = Raffinose D.T., OXI = Oxidase D.T., α-GAL = Alpha-Galactosidase D.T., MAN = Mannitol D.T., ONPG = ONPG.D.T., MAL = Maltose D.T., GEL = gelatinase, ADH = Arginine Dihydrolase. D.T., LAP = Leucine Aminopeptidase D.T., RHAM = l-Rhamnose D.T., ARG=Arginine Aminopeptidase D.T.

6) <u>Pigmented</u> gram negative rods (anaerobes)(Kana R, Oxgcall <u>S</u>, Metro S, Red fluorescence, CAT <u>0</u>)

	IND	α-GLU	ONPG	α-FUC	ARG	NAG	LIP	Remarks
Prev. denticola/ loeschei	0	+	+	+	0	+	+	Vanco R
P.melaninogenica					+			
Prev. intermedia/nigrescens	+	+	0	+	+	0	+	Vanco R
Prev. pallens	+	+	0	+	+	0	0	Vanco R
Prev. corporis	0	+	0	0	·	0	0	Vanco R
Prev. bivia	0	+	+	+	+	+	0	Vanco R, RAF 0
Prev. disiens	0	+	0	0	$+^{0}$	0	0	Vanco R
Prev. multiformis	0	+	+	$+^{0}$	+	·	0	Vano R, RAF +
Porph. asaccharolytica	+	0	0	+	·	0	0	TRYP 0, Vanco S
Porph. gingivalis	+	0	+	0	V	+	0	TRYP +, Vanco S
Porph. endodentalis	+	0	0	0	+	0	0	TRYP 0, Vanco S
Prev. massiliensis (non-pigm.)	+	0	0	0	0	·	·	Vanco R, Kana S, OXI+
Odoribacter splanchnicus	+	0	0	+	·	+	·	PYR+, Vanco R

IND = Indole D.T., α-GLU = Alpha-Glucosidase D.T. α-FUC = Alpha-Fucosidase D.T., NAG = Beta-N-Acetylglucosaminidase D.T., LIP = Lipase, TRYP = Trypsin D.T., Vanco = Vancomycin 5 µg Neo-S (S ≥ 20 mm, R ≤ 18 mm), RAF = Raffinose D.T.

7) Differentiation of Bacteroides/Parabacteroides (5)

	α-FUC	IND
Bacteroides (IND 0)	+	0
Bacteroides (IND +)	V	+
Parabacteroides	0	0

α-FUC = Alpha-Fucosidase D.T., IND = Indole D.T.

8) Differentiation of Parabacteroides (5,6) (OXG R, Vanco R, Kan 500 R, Col R)

	α-FUC	IND	ß-GLU	PGUA	PYR	CAT	Remarks
P. distasonis	0	0	+	0	0	+	
P. merdae	0	0	0	+	+	0	
P. goldsteinii	0	0	+	+	0	V	ß-XYL +
P. johnsonii	0	0	0	+	0	+	

α-FUC = Alpha-Fucosidase D.T., IND = Indole D.T., ß-GLU = Beta-Glucosidase D.T, PGUA = Beta-Glucuronidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., CAT = catalase.

9) Differentiation of Streptococcus suis, within the S. mitis group (VP neg)

	ADH	α-FUC	PGUA	ESC	TRE	Remarks
S. suis	V	+	$+^{0}$	+	+	CAMP+, OPT R
S. gordonii	+	+	0	+	+	B-MAN+
S. sangninis	+	0		+	+	
S. parasanguinis	+	V		0	0^{+}	
S. mitis	V	0		0	0	
S. oralis	0	0		0	V	
S. sinensis	+	·	0	+	+	VP+, BE+

In regions with pig farming industries, S. suis should be considered if the strain is optochin resistant and VP negative. Streptococci are cultured from a CSF sample obtained from a patient with meningitis.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Alpha-Fucosidase (p-Nitrophenyl-α-L-Fucosidase)	*B. fragilis* ATCC 25285	*E. coli* ATCC 25922

References (α-FUC)

1) Heltberg et al: The cultivation and rapid enzyme identification of DF-2. Eur. J. Clin. Microbiol. **3**, 241-3, 1984.
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6) Sakamoto M. et al: Parabacteroides johnsonii sp. nov., isolated from human faeces. Intl. J. System Evol. Microbiol. **57**, 293-296, 2007.

3.20.3 BETA-FUCOSIDASE (ß-FUC)

REF No. 59921

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	+	+	$+^0$	0	·	
subsp. *Pharyngis*						
S. intermedius	+	+	V	0^+	+	
S. sinensis	·	0	+	0	·	BE+. αGAL0, AlkP0

NAG = Beta-N-Acetylglucosaminidase D.T., ß-GLU = Beta-Glucosidase, RAF = Raffinose D.T., α-GLU = Alpha-Glucosidase D.T.

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 *	0	+	V	0		0		+
subsp. *Equisimilis (ACG)*								
S. constellatus	+	0	$+^0$	+		0		·
subsp. *pharyngis*								
S. equi	0	+	V	0	+	+	0	0
subsp. *zooepidemicus (C)*								
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 D.T., PGUA = Beta-Glucuronidase D.T., ß-GLU = Beta-Glucosidase D.T., ß-FUC Beta-Fucosidase D.T., SORB = Sorbitol D.T., TRE = Trehalose D.T.

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.

3.20.4 ALPHA-GALACTOSIDASE (α-GAL)

REF No. 50211

Results

1) Nutritionally variant streptococci (NVS) = *Abiotropia*, *Granulicatella* spp and Helcococcus spp. (PYR +, LAP+)

	α -GAL	PGUA	NAG	ADH	PYR	ONPG
A. defectiva	+	0	0	0	+	+
G. adiacens	0	+	0	0	+	0
G. elegans	0	0	0	+	+	0
G. balaenopterae	0	0	+	+	·	
Helcococcus kinzii	0	0	·	0	+	+

α -GAL = Alpha-Galactosidase D.T., PGUA = Beta-Glucuronidase D.T., NAG = Beta-N-acetylglucosaminidase D.T. and ADH = Arginine Dihydrolase D.T., LAP = Leucine Aminopeptidase D.T.

2) Anaerobe gram negative rods pigmented (Oxgall S, Kana R, Metro S, Red fluoroscene, CAT0, Brilliant Green S, α-GLU +, TRYP 0)

	α-GAL	α-FUC	IND	CEL	LIP
Prevotella melaninogenica	+	+	0	0	+
P. intermedia/nigrescens	0	+	+	0	+
Prevotella denticola	+	+	0	0	+
Prevotella loescheii	+	+	0	+	+
Prevotella corporis	0	0	0	0	0
Prevotella pallens	0	+	+	0	0

α GAL = Alpha-Galactosidase D.T., α -FUC = Alpha-Fucosidase D.T., IND = Indole D.T. and CEL = Cellobiose D.T (add 3 drops of paraffin oil), LIP = Lipase Oxgall D.T. (S ≥ 10 mm, R < 10 mm), Brilliant green D.T. (S ≥ 10 mm, R < 10 mm), α-GLU = Alpha-Glucosidase D.T., TRYP = Trypsin D.T.

3) Differentiation of Aerotolerant *Clostridium* spp. (Vanco S, Kana S, Col R)

	α-GAL	ONPG	PYR	Gel	SORB	HEM
Clostridium tertium	+	+	0	0	0	α/ß
Clostridium histolyticum	+	0^{+}	+	+	0	0
Clostridium intestinale	·	·	·	0	+	beta

α-GAL = Alpha-Galactosidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., Gel = Gelatine hydrolysis, SORB = Sorbitol, HEM = hemolysis.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Alpha-Galactosidase (p-Nitrophenyl- α-D-Galactopyranoside 0.3)	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853

References (α-GAL)

1) Ruoff K.L.: Nutritionally variant streptococci. Clin. Microbiol. Reviews **4**, 184-90, 1991.
2) Steyaert S. et al: Septicemia in neutropenic patients infected with Clostridium tertium resistant to Cefepime and other expanded-spectrum cephalosporins, J. Clin. Microbiol. **37**, 3778-9, 1999.
3) Christensen J.J., Facklam R.R.: Granulicatella and Abiotrophia species from human clinical specimens. J. Clin. Microbiol. **39**, 3520-3, 2001.

3.20.5 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 D.T., URE = Urease D.T., IND = Indole D.T., NAG = Beta-N-Acetylglucosaminidase D.T. and PZA = Pyrazinamidase D.T.

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 D.T., OXI = oxidase, CAT = catalase, SUC = Sucrose D.T., α-GLU = Alpha-Glucosidase D.T., TRYP = Trypsin D.T., IND = Indole D.T., NIT = Nitrate reduction, ALA = Porphyrin D.T., Col = Colistin 10 µg D.T. ($S \geq 13$ mm, $R \leq 10$ mm), α-FUC = Alpha-Fucosidase D.T.

* *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 ssp. *haemolyticus* (B-11)	+	+wk	+	V	$+^0$	$+^0$	V	+	0^+	ß-haem $+^0$
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 D.T., CAT = Catalase, αGAL = Alpha-Galctosidase D.T., αGLU = Alpha-glucosidase D.T., ßXYL = Beta-Xylosidase D.T., ßGLU = Beta-Glucosidase D.T., SOR = Sorbitol D.T., TRE = Trehalose D.T., MAN = Mannitol D.T., ß-haem = beta haemolysis, LACT = Lactose D.T., URE = Urease D.T., $+^R$ = rapidly positive, IND = Indole D.T., ODC = Ornithine Decarboxylase D.T.

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.

3.20.6 ALPHA GLUCOSIDASE (α-GLU) BETA-GLUCOSIDASE (ß-GLU)

REF No. 50411
REF No. 50511

Results

1) *Gardnerella vaginalis* and Atopobium vaginae

CAT 0, OXI 0

	α-GLU	ß-GLU	SPS	HIP	Remarks
Gardnerella vaginalis	+	0	S (≥10 mm)	+	PRO +
Lactobacillus vaginalis	+	·	R	V	
Corynebacteria vaginalis	V	·	R	V	
Bifidobacterium	+	·	R	0	Gentamicin R
Atopobium vaginae	0	0	R	·	PRO +, ADH +, LAP +, Metro R, Vanco S.

2a) *Enterobacter (Cronobacter) sakazakii* (2,3) PYR+, βXYL+

	α-GLU	TRIB
Cronobacter sakazakii	+	+
Enterobacte cloacae	0	0
Enterobacter aerogenes	0	
Pantoea agglomerans	0	

α-GLU = Alpha-Glucosidase D.T., α-GLU = Beta-Glucosidase D.T., SPS = S.P.S. D.T., HIP = Hippurate Hydrolysis D.T.

2b) Enterobacter (Cronobacter) sakazakii and similars (5) (VP+, α-GLU+)

Recently E. sakazakii changed to Cronobacter with several species, all α-GLU positive
They currently show Roscozym codes 5460 and 5462 and may be differentiated as follwos:

	IND	DUL	MALON
Cronobacter sakazakii	0	0	0
Cronobacter muytjensii	+	+	
Cronobacter dublinensis	+	0	
Cronobacter turicensis	0	+	
Cronobacter malonaticus	0	0	
Cronobacter genomospecies 1	0	+	+

IND= Indole D.T, DUL=Dulcitol and MALON=Malonate

2c) Differentiation of Cronobacter spp from Enterobacter spp (PYR +)

	α-GLU	VP	ADH	SORB	SUC
Cronobacter spp	+	+	+	0	+
E. aerogenes	0	+	0	+	+
E. clocae	0	+	+	+	+
Other Enterobacter spp	0	$+^{0}$	$+^{0}$	0^{+}	$+^{0}$
E. helveticus/tuvicensis	+	0	0	0	0
E. pulveris	+	0	0	0	+

3) Differentiation of *C. albicans* from *C. dublinensis*

	α-GLU (2h)
Candida albicans	+
Candida dublinensis	0

α-GLU (2h) = Alpha-Glucosidase D.T. (incubation 2 hours).

4) Phenotypic patterns of Aerococcus urinae (4)

	ß-GLU	PGUA	ESC
A. urinae phenotype I	0	+	0
A. urinae phenotype II	+	0	+

ß-GLU = Beta-Glucosidase D.T., PGUA = Beta-Glucosidase D.T., ESC = Esculin Hydrolysis D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Alpha-Glucosidase (p-Nitrophenyl-α-D-Glucopyranoside)	*S. maltophilia* ATCC 13637	*P. aeruginosa* ATCC 27853
Beta-Glucosidase (p-Nitrophenyl-ß-D-Glucopyranoside)	*K. pneumoniae* ATCC 13883	*Morganella morganii* ATCC 25830

References (α-GLU, ß-GLU)

1) Bastida Vilá M.T. et al: Gardnerella vaginalis bacteremia in an adult male. J. Clin. Microbiol. Infect. Dis. **16**, 400-1, 1997.
2) Muytiens H.L. et al: Enzymatic profiles of Enterobacter sakazakii and related species with special reference to α-glucosidase reaction and reproducibility of the test system. J. Clin. Microbiol. **20**, 684-6, 1984.
3) Poterac. E. sakazakii unexpectedly widespread in some food-processing plants. ASM news, **70**, 109, 2004.
4) Christensen J.J. et al: Aerococcus urinae: polyphasic characterisation of the species. APMIS **113**, 517-525, 2005.
5) Iversen C. et al: The taxonomy of E. sakazakii: proposal of a new genus Cronobacter gen. nov. and descriptions of Cr. sakazakii comb. nov. Cr. sakazakii subsp sakazakii comb. nov. Cr. sakazakii subsp malonaticus subsp nov, Cr.turicensis sp. nov Cr.muytjensis nov, Cr. dublinensis sp nov and Cr. genomspecies I. BMC Evol. Biol. **7**, 64-74, 2007.

3.20.7 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: Colorless

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 D.T., SUC = Sucrose D.T.

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.

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

Quality Control

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

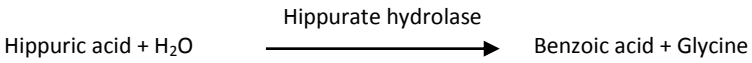
References (ß-XYL)

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.

3.21.0 HIPPURATE HYDROLYSIS (HIP)

REF No. 56711

Diagnostic Tablets for determinating 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: Colorless, 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 color 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 subsp. *dysgalactiae* (C)	0	0^+	+	0	0
S. uberis (E, P, U, G)	$+^0$	+	+	+	0^+
S. parauberis	$+^0$	+	0	+	0
S. canis (G)	0	$+^0$	0^+	V	+

ESC = Esculin Hydrolysis D.T., PGUA = Beta-Glucuronidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., 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 D.T., 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 D.T., PGUA = Beta-Glucuronidase D.T., α-GAL = Alpha-Galactosidase D.T., ADH = Arginine Dihydrolase D.T., SUC = Sucrose D.T., TRE = Trehalose D.T.

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 D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., HIP = Hippurate Hydrolysis D.T., INU = Inulin D.T., RAF = Raffinose D.T.

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 D.T., α-FUC = Alpha-Fucosidase D.T., α-GLU = Alpha-Glucosidase D.T.

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.
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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.
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3.22.0 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 color (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: Colorless, slightly colored 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 human blood (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 0
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 D.T., HIP = Hippurate Hydrolysis D.T., NO_3 = Nitrate Reduction D.T., URE = Urease D.T., GLU = Gamma-Glutamyl Aminopeptidase D.T., NAL = Nalidixan Neo-S, (S>16 mm R<16mm), CLTN = Cephalothin Neo-S (S>16mm R<16mm), AlkP = Alkaline Phosphatase D.T., CAT = catalase, $+^R$ = rapid positive, PZA = Pyrazinamidase D.T., 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 D.T., NO_3 = Nitrate Reduction D.T., OXI = Oxidase D.T., 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 cost effective protocol for the isolation of <u>all</u> 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 D.T., Growth McC = Growth in McConkey, NAL = Nalidixan Neo-S, CLTN = Cephalothin Neo-S, Col = colistin. NO_3 = Nitrate Reduction D.T.

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.
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3.23.0 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 color 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 color 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 over layer 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 D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., DEFRX = Deferoxamine D.T., LDC = Lysine Decarboxylase D.T.

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.

3.24.0 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 D.T.α-GLU = Alpha-Glucosidase D.T., IND = Indole D.T., PRO Proline Aminopeptidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., Alkaline Phosphatase D.T., SPS = SPS D.T.

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.

3.25.0 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 D.T., SPS = SPS D.T., □-GLU = Alpha-Glucosidase D.T., ß-GLU = Beta-Glucosidase D.T., HIP = Hipurate Hydrolysis D.T.

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.

3.26.0 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: Colorless, 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 D.T., NO_3 = Nitrate Reduction D.T., TRIB = Tributyrin D.T., COL = Colistin 10 µg D.T. (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-α-Naphthylamine sol.** and **Sulfanilic acid sol.** is added to the tablet.

Reading of the test

A **pink or red** color 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 D.T., IND = Indole D.T., ß-XYL=Beta-Xylosidase D.T. and CAT = catalase.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
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.
2) Foster G. et al: Staph. lutrae sp. nov., a new coagulase-positive species isolated from otters. Intl. J. Syst. Bacteriol. **47**, 724-6, 1997.
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.

3.27.0 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 D.T. (S ⍰16mm, R ⍰14mm), FOSFO = Fosfomycin Neo-S, MSE = Mannose D.T.

3) Anaerobes

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

A. prevotii, A. tetradius	

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 D.T.

References

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3.28.0 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 cannot reliably be tested with O/129.

ODC = Ornithine Decarboxylase D.T., LDC = Lysine Decarboxylase D.T., ADH = Arginine Dihydrolase D.T., ARA = Arabinose D.T., TRYP = Trypsin D.T., MAN = Mannitol D.T.

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 D.T., Alk P = Alkaline Phosphatase D.T., 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 D.T., NAG = Beta-N-Acetylglucosaminidase D.T., LAP = Leucine Aminopeptidase D.T., MAL = Maltose D.T., PZA = Pyrazinamidase D.T., AMP = Ampicillin 33 µg Neo-S, ADH = Arginine Dihydrolase D.T., OCT = Ornithine Decarboxylase D.T., α-GLU = Alpha-Glucosidase D.T. 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

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

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 D.T., ADH = Arginine Dihydrolase D.T., 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

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3.30.0 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 positive microorganisms are:

Neisseria	Aeromonas	Pasteurella
Vibrio	most *Pseudomonas* spp.	Flavobacterium
Alcaligenes	Moraxella	Campylobacter
Plesiomonas.		

Among the oxidase negative 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 D.T., 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.

3.31.0 POLYMYXINS 150 µg (CO150) Neo-Sensitabs

REF No. 77512

Polymyxins 150 µg (Colistin) Neo-Sensitabs are useful in identification of staphylococci. ***S. aureus*** (zones ≤12 mm) is the most resistant species, but some strains of *S. epidermidis*, *S. hyicus* and a few strains of *S. lugdunensis* may also show small zones of inhibition (≥13 mm).

Procedure

Sensitivity testing of staphylococci is performed on Mueller-Hinton II agar without blood using McFarland 0.5 inoculum. Danish Blood Agar may be used with semiconfluent growth. Polymyxins 150 µg Neo-Sensitabs may be added to the routine antibiogram. Incubate at 35 °C overnight.

Results

1) Staphylococci

	McFarland 0.5 (Kirby- Bauer)		Semi-confluent growth
		Resistant	
S. aureus	≤ 12 mm		≤ 15 mm
		Sensitive	
Other staphylococci	≥ 14 mm		≥ 16 mm

2) Non-fermenters

	Poly
Shewanella algae	R (< 18 mm)
Shewanella putrefaciens	S (≥ 20 mm)

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

	CAT	GLU	NO_3	TRIB	POLY	Pigment	Remarks
N. elongata subsp. *clycolytica*	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 (7)	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 D.T., NO_3 = Nitrate Reduction D.T., TRIB = Tributyrin D.T., POLY = Polymyxins 150 µg Neo-S (S ≥ 20 mm, R < 18 m).

Quality Control (McF 0.5)

NEO-SENSITABS	Code	***E. coli*** **ATCC 25922**	***Ps. aeruginosa*** **ATCC 27853**
Polymyxins 150 µg	CO150	19-24 mm	20-25 mm

References

1) Heltberg O., Bruun B.: Polymyxin susceptibility in staphylococci differentiating coagulase positive and coagulase negative strains. Acta path. microbiol. immunol. Scand., Sect. B, **91**, 157-161, 1983.
2) Heltberg O., Bruun B.: Recognition of coagulase negative Staph. aureus strains by primary polymyxin usceptibility testing. Acta path. microbiol. immunol. Scand., Sect. B, **92**, 115-118, 1984.
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3.32.0 PORPHYRIN (d-Ala) (ALA)

REF No. 57321

Contain delta-aminolevulinic acid for the detection of hemin (X-factor) requirement in the **differentiation of *Haemophilus influenzae* from *Haemophilus parainfluenzae***. This enzymatic test is rapid and independent of several factors affecting the usual tests for growth factor requirements (e.g. presence of X-or V-factor in the test medium, "carry over" of X-factor with the inoculum from chocolate agar, lack of other essential nutrients in the test medium).

Principle of the Test

Haemophilus parainfluenzae does not require hemin (X-factor) for growth because it possesses enzymes for the biosynthesis of heme (Fig. 1). When supplied with delta-aminolevulinic acid, *Haemophilus parainfluenzae* strains synthetize porphobilinogen and porphyrins, which are detected in the test.

Porphyrins show characteristic **red fluorescence** when exposed to long wave UV-light (360 nm). Porphobilinogen that contains a pyrrole ring produces a **red colour with Kovacs' reagent** (92031) (in the lower water phase).

Kilian (1974) tested 134 Haemophilus strains and found a perfect agreement between the two methods of reading.

delta-Aminolevulinic acid
⇓
Porphobilinogen
⇓
Porphyrins
⇓
Protoporphyrin IX
⇓
Heme

Fig. 1. Main steps of the heme biosynthesis.

Haemophilus influenzae that requires hemin for growth lacks the enzymes for heme synthesis and consequently does neither produce porphyrins nor porphobilinogen from delta-aminolevulinic acid (negative reaction).

Procedure

Prepare a dense bacterial suspension (at least McFarland No. 4) of fresh colonies (18-24 hours) of the haemophilus strain to be tested in 0.25 ml saline in a tube. Add one Porphyrin (ALA) diagnostic tablet and close the tube. Incubate at 35-37 °C for **4-6 hours** or in case of negative or doubtful reactions for **up to 24 hours**.

Reading of the test

The test can be read in two ways:

a) Add **4 drops of Kovacs' reagent** (92031), shake and wait for up to 10 minutes.

Positive reaction:	**Red/pink** color in the **lower water phase**
The strain does not require X-factor:	***Haemophilus parainfluenzae***
Negative reaction:	**Colorless** water phase
The strain requires X-factor:	***Haemophilus influenza***

After the addition of Kovacs' reagent the tube cannot be reincubated for re-reading. For rapid results, it is advisable to incubate two tubes for each strain: one for addition of reagent after 4 hours, and another for later confirmation (re-incubation), if a negative test result is obtained.

b) Expose to long wave UV light, 360 nm (Wood's lamp).

Positive reaction:	**Red fluorescence**
The test strain does not require X-factor:	***Haemophilus parainfluenzae***
Negative reaction:	**No red fluorescence**
The test strain requires X-factor:	***Haemophilus influenzae, Haemophilus haemolyticus***

In case of doubtful or negative reactions, the tube should **be re-incubated for 18-24 hours**.

Other haemophilus

	ALA
H. ducreyi (usualliy genital sources)	0
H. aegyptius (conjunctivitis)	0
H. haemolyticus (oral flora)	0
Aggregatibacter aphrophilus	+ or 0
Aggregatibacter segnis	0

Other bacteria

	ALA (Kovacs')
Staphylococci (CAT +)	+
Staphylococci (CAT 0)	+
Streptococci	0
Aerococci	0
Rothia mucilaginosa	+

ALA = Porphyrin D.T.

1) Differentiation of *Aggregatibacter* spp. (ALA +^{0}, NO_3 +, Alk P +, non-haem)

	ALA	**CAT**	**ONPG**	**SUC**	**TRE**	**γ-GLU**	**LACT**
A. actinomycetemcomitans	+	+	0	0	0	+	0
A. aphrophilus	+/0	0	+	+	+	+	+
A. segnis	+	V	V	+	0	0	0
Haemophilus parainfluenzae	+	.	.	+	0	0	0

CAT = Catalase, ONPG = ONPG D.T. SUC = Sucrose D.T., TRE = Trehalose D.T., γ-GLU = Gamma-Glutamyl Aminopeptidase D.T.

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Porphyrin (d-Ala) (d-Aminolevulinic acid)	*H. parainfluenzae* ATCC 7901	*H. influenzae* ATCC 49247

References

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3.33.0 PS. AERUGINOSA SCREEN (PSAER)

REF No. 59311

Keeven and DeCicco (1989) found that 1,10-phenanthroline has a high selective specificity for *Pseudomonas aeruginosa*.

Ps. aeruginosa Screen Diatabs contain 80 µg diffusible amount per tablet and are useful for the presumptive identification of *Pseudomonas aeruginosa*.

Procedure

Place one Ps. aeruginosa Screen Diagnostic Tablet on an inoculated plate (Mueller-Hinton agar or similar) for sensitivity testing. Incubate at 35-37 °C for **18-24 hours**.

Read the diameter of the inhibition zone in mm. Measure **only the clear zone** with no growth.

Reading of the test

	PSAER	
	10^8 CFU/ml Confluent growth (Kirby-Bauer)	**10^5-10^6 CFU/ml Semi-confluent growth**
Pseudomonas aeruginosa	≤14 mm	≤16 mm
Other Pseudomonas species, non-fermenters, and Enterobacteriaceae	≥18 mm	≥20 mm

Results

1) Strains from cystic fibrosis patients

	PSAER	COL	PYR	TRYP	TRIB	Remarks
Ps. aeruginosa	R	S^R	$+^0$	+	$+^0$	
Burk. cepacia complex	S	R^S	0	0	+	ADH 0, GLU + Genta R
Achr. xylosoxidans	S	V	+	0	0	Aminoglyc R, Quinolones R
St. maltophilia	S	V	0	+	+	
Inquilinus limosus	S	R	+	+	·	Res, beta lactams, except imipenem
Pandoraea spp.	S	R	0	0	$+^0$	Meropenem R, URE +, Alk P +, Genta/Tobra R, LAP +, MOT +, CAT +, LCD 0, ODC 0, ONPG 0

COL = Colistin 10 µg D.T. (S ≥ 13 mm, R ≤ 10 mm), PYR = Pyrrolidonyl Aminopeptidase D.T., TRYP = Trypsin D.T., S^R = most strains are S, R^S = most strains are R, res = multiresistant, TRIB = Tributyring D.T.

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
Ps. aeruginosa Screen 80 µg (1,10 Phenanthroline)	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853

References

1) Keeven J.K., De Cicco B.T.: Selective medium for Pseudomonas aeruginosa that uses 1,10-Phenanthroline as the selective agent. Appl. Environm. Microbiol. **55**, 3231-3233, 1989.
2) Pringler N., Casals J.B.: Identification of Pseudomonas aeruginosa with Ps. aeruginosa Screen Diagnostic Tablets, 5th European Congress of Clinical Microbiology and Infectious Diseases, Oslo, Sep., 1991.
3) Fijita S. et al: Identification of Ps. aeruginosa by using a disk of phenanthroline and C-390 and by cell agglutination testing with monoclonal antibodies. J.Clin. Microbiol. **30**, 2728-9, 1992.

3.34.0 PYRAZINAMIDASE (PZA)

REF No. 59811

Test to differentiate pathogenic corynebacteria (negative reaction) from other corynebacteria (positive reaction). The enzyme pyrazinamidase catalyzes the hydrolysis of pyrazinamide into pyrazinoic acid and ammonia. Also, useful in the differentiation of *Yersinia enterocolitica* serotypes.

Procedure

Make a turbid, "milky" suspension equivalent to at least McFarland No. 8 (Corynebacteria) or No. 4 (Yersinia) of the test strain from an agar plate culture in 0.25 ml distilled water in a tube. Add one PZA Diatabs and close the tube. Incubate for **4 hours** or **over-night** at 35-37 °C.

Reading of the test

After incubation add one drop of ferrous ammonium sulphate solution 5% w/v in purified water (freshly prepared or stored at -20°C).

Positive reaction: **Orange, red**
Negative reaction: Colorless, light yellow

Results

1) Corynebacteria

The pathogenic species *C. diphtheriae*, *C. pseudotuberculosis* and *C. ulcerans* give a negative reaction, while other corynebacteria give a positive or variable reaction.

2) *Yersinia enterocolitica* (5)

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

PZA = Pyrazinamidase D.T., SAL = Salicin D.T., ESC = Esculin Hydrolysis D.T. All tests performed at **25 °C**.

3) *Yersinia enterocolitica* and *Y. enterocolotica*-like nontipeable species (6)

	COL on CIN agar	ESC	SAL	PZA
Yersinia enterocolitica	No ground glass appearance	V	V	0^{+}
Yersinia enterocolitica-like	Erose edges			
a) *Y. mollaretti*	and ground glass	0	0	+
b) Y. bercovieri	appearnce	0	0	+

Col on CIN agar = colony morphology on CIN–agar. All test at 25 °C.

4) Differentiation of Brevibacterium/Arthrobacter. Large colony forming, whitish/greyish, non-cheese like smelling, non-fermentative gram positive rods, CAT+, LAP+, Fosfomycin R

	NO_3	TRIB	NAG	AlkP	αGLU	ONPG	PZA	PYR	TRYP	DEFER	Remarks
Brev.casei	V	·	0	+	+	0	+	V	·	-	PRO+, cheese smell O/129 R, Fura R
Brev. luteolum	0	·	+	0^+	0	0	+	+	·	-	
Brev.otitidis	0	·	0	·	0	0	+	+	·	-	
Brev.paucivorans	0	0	$+^0$	·	0	0	0	0	·	-	
Brev. ravenspurgense	0	+	0	V	0	0	+	0	V	-	
Arthrob. albus	0	·	0	+	0	0	·	+	+	R	
Arthr.cumminsii	0	+	0	·	0	0	·	+	·	S	
Arthr. oxydans	+	·	0	·	+	+	·	0	·	R	
Arthr. sanguinis	0^+	+	+wk	+	+	0	+	V	+	·	αMAN+, URE 0
Arthr. woluwensis	0	+	+	+	+	+	·	+	+	R	αMAN+, URE +

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Pyrazinamidase (Pyrazinamide)	*E. coli* ATCC 25922	*S. aureus* ATCC 25923

References

1) Colman G., Weaver E., Efstratiou A.: Screening tests for pathogenic corynebacteria. J. Clin. Pathol. **45**, 46-48, 1992.
2) Farmer III J.J. et al: Pyrazinamidase, CR-MOX Agar, Salicin fermentation Esculin hydrolysis and D-xylose fermentation for identifying pathogenic serotypes of Yers. enterocolitica. J. Clin. Microbiol. **30**, 2589-94, 1992.
3) Efstratiou A., Sesardic D., Hoy C.S., Sangrador A., Cookson B.D.: Microbiological Diagnosis of Diphtheria. Poster 448, 6th European Congress of Clinical Microbiology and Infectious Diseases, Seville, Spain, March, 1993.
4) Efstratiou A.: Laboratory Diagnosis of Diphtheria (European Region WHO). Pyrazinamidase Test and Rosco Identification System, pages 9-10, 37-42, 1994.
5) Chiesa C. et al: Identification of pathogenic serotypes of Yersinia enterocolitica. J.Clin. Microbiol. **31**, 2248-50, 1993.
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3.35.0 S.P.S. (SPS)

REF No. 44611

SPS Diatabs contain 1 mg diffusible amount of sodium polyanethol sulfonate per tablet and are useful for identification of ***Peptostreptococcus anaerobius*** and ***Gardnerella vaginalis***.

Procedure

SPS diagnostic tablets are placed on inoculated blood agar plates (inoculum equivalent to McFarland 0.5 - confluent growth) before incubation.

1) ***Peptostreptococcus anaerobius*** is sensitive to SPS and a zone of inhibition around the diagnostic tablet is produced (≥12 mm). Incubate anaerobically for 48 hours at 35-37 °C.

   Other *Peptostreptococcus* species are resistant to SPS (no zone of inhibition).

   Vanco S, Col R, Metro S

	SPS	GLU	α-GLU	IND	PRO	PYR	Alk P	Remarks
P. anaerobius	S (≥ 12 mm)	+	+	0	+	0	0	
P. stomatis	S (≥ 15 mm)	+	+	0	0	0	0	
Peptoniphilus asaccharolyticus	R	0	0	+^{0}	0	0	+	
Peptoniphilus harei	R	0	0	0	0	0	0	
P. gorbachii	R	0	0	V	·	0	0	LAP+, COL R
P. olsenii	R	0	0	V	·	0	+	LAP+, COL S
Parvimonas micra	V	0	0	0	+^{0}	+	+	
F. magna	R	0	0	0	0	+	V	
A. murdochii	R	+	·	0	0	+	+	ONPG+,ADH+,LAP+
Anaerococcus prevotti	R	0wk	+	0	0	+^{0}	V	URE+, PGUA+
Anaerococcus vaginalis	R	+	V	0	0	0	V	LAP +, ADH+
Gallicola barnesae	R	0	0	wk	0	0	0	

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

   Use FAA + 5% blood or Brucella Blood Agar **and incubate in anaerobic atmosphere**.

2) ***Gardnerella vaginalis*** is sensitive to SPS and shows an inhibition zone of ≥ 10 mm.

   Lactobacillus spp., *Corynebacterium* spp., *Bifidobacterium* spp., and vaginal streptococci are resistant.

	SPS	HIP	α-GLU	ß-GLU	Remarks
Gardnerella vaginalis	S (≥ 10 mm)	+	+	0	CAT 0, OXI 0, PRO +
Vag. lactobacilli	R	V	+	·	
Vag. corynebacteria	R	V	V	·	
Bifidobacterium	R	0	+	·	
Atopobium vaginae	R	·	0	0	PRO +, ADH +, LAP +, Metro R, Vanco S

   HIP = Hippurate hydrolysis D.T., ß-GLU = Beta-Glucosidase D.T.

   Use Mueller-Hinton II agar + 5% blood and **incubate in an atmosphere with 5-10% CO_2**.

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
S.P.S. 1000 µg (Sodium polyanethol sulfonate)	*G. vaginalis* ATCC 14018	*Kocuria rhizophila* ATCC 9341

References

1) Graves M.H., Morello J.A., Kocka F.E.: Sodium polyanetholsulfonate sensitivity of anaerobic cocci. Appl. Microbiol. **27**, 1131-1133, 1974.
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4) Murdoch D.A.: Gram-positive anaerobic cocci. Clin. Microbiology Reviews **11**, 81-120,1998.
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3.36.0 SUGAR FERMENTATION Tests (SFT)

Diatabs for sugar fermentation contain the specific sugar substrate together with a weak buffer and an indicator (phenol red), which changes color from red to **yellow** in case of a **positive** reaction.

Range

The range of sugar fermentation tests comprises:

Diatabs	**Code**	**REF No.**
Adonitol	(ADO)	(52011)
l-Arabinose	(ARA)	(52121)
Cellobiose	(CEL)	(non-stock)
Dulcitol	(DUL)	(non-stock)
Fructose	(FRU)	(non-stock)
Galactose	(GAL)	(non-stock)
Glucose	(GLU)	(52611)
Inositol	(INO)	(non-stock)
Inulin	(INU)	(52711)
Lactose	(LAC)	(52811)
Maltose	(MAL)	(52911)
Mannitol	(MAN)	(53011)
Mannose	(MSE)	(53111)
Melibiose	(MEL)	(53211)
Raffinose	(RAF)	(53311)
l-Rhamnose	(RHAM)	(53411)
Ribose	(RIB)	(non-stock)
Salicin	(SAL)	(non-stock)
Sorbitol	(SOR)	(53711)
Sucrose	(SUC)	(53811)
Trehalose	(TRE)	(53911)
d-Xylose	(XYL)	(54021)

Procedure

Please note: Colonies should be obtained from bacteria grown on sugarfree media, such as TSA agar.
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 sugar diagnostic tablet and close the tube. Incubate at 35-37 °C for **4 hours** or **overnight**.

Positive reaction: **Yellow**, yellow-orange (4 hours), **yellow** (overnight)
Negative reaction: Red, orange-red

For identification of pathogenic Neisseria we recommend the use of enzymatic tests (Gamma Glutamyl aminopeptidase (46711), Tributyrin (48821), ONPG (50311) and the Superoxol test).

Please note:

For sugar fermentation tests with anaerobes, add 3 drops of paraffin oil before incubation.

Results

1) Differentiation of Yersinia

	SUC	RHAM	RAF	VP (25°C)	IND	LDC	ODC
Yersinia enterocolitica	$+^0$	0	0	+	V	·	·
Yersinia frederiksenii	+	+	0	+	+	·	·
Yersinia intermedia	+	+	+	+	+	·	·
Yersinia kristensenii	0	0	0	0	V	0	+
Yersinia aleksiciae	0	0	0	·	·	+	+
Yersinia aldovae	0	+	0	+	0	·	·
Yersinia bercovieri	+	0	0	0^+	0	·	·
Other *Yersinia* spp.	V	0	V	0	0	·	·

SUC = Sucrose D.T., RHAM = Rhamnose D.T., RAF = Raffinose D.T., VP(25°C) = Voges Proskauer D.T (at 25° C), IND = Indole D.T., TRE = Trehalose D.T., LDC = Lysine Decarboxylase D.T., ODC = Ornithine Decarboxylase D.T.

2a) *Yersinia enterocolitica*

	SUC	PZA	VP
Yersinia enterocolitica (path.)	+	0	+
Yersinia enterocolitica (path. atypical)	0	0	+
Y. enterocolitica BT1A	+	+	+ (non-pathogenic)
Yersinia kristensenii	0	+	0

SUC = Sucrose D.T., PZA = Pyrazinamidase D.T. and VP = Voges Proskauer D.T.
All tests performed at **25 °C**.

2b) Pathogenic Y. enterocolitica biotypes (14)

	Lipase	XYL	PZA	ESC	TRE	IND
Y. enterocolitica BT1B	+	+	0	0	+	+
Y. enterocolitica BT2	0	+	0	0	+	+
Y. enterocolitica BT3	0	+	0	0	+	0
Y. enterocolitica BT4	0	0	0	0	+	0
Y. enterocolitica BT5	0	0	0	0	0	0
Y. enterocolitica BT1A (non-pathogenic)	$+^0$	+	$+^0$	+	+	+

3) Enterococci resistant to vancomycin (7,8)

	RM	PIGM	ARA	XYL®	FURAZ	Remarks
Enterococcus faecalis	0	0	0	0	S	MAN+, SORB+
Enterococcus faecium	0	0	100	0	R	
Enterococcus casseliflavus	96	95	92	·	S	
Enterococcus gallinarum	100	0	90	+	S	
Enterococcus sanguinicola	0	0	0	·	·	MAN+, SORB 0

RM = Rapid motility (incub. 4 h at 35 °C), PIGM = Pigment, ARA = Arabinose D.T., XYL® = Rapid Xylose (incub. 2 h at 37 °C, McF 3) (8), FURAZ = Furazolidone Neo-S.

4) Differentiation within the *Streptococcus bovis gallotytitus*

	MAN	NAG	αGAL	PGUA	Remarks
Streptococcus bovis (bio II)	0	0		0	
S. gallolyticus (bovis bio I)	$+^0$	$+^0$	0	0	Colonic cancer
S. infantarius (bio II/I)	0	0	+	0	Non-colonic cancer, VP+
S. pasteurianus (bio II)	0	0	+	+	

MAN = Mannitol D.T. and NAG = Beta-N-Acetylglucosaminidase D.T., αGAL=Alpha Galactosidase DT. PGUA=Betaglucuronidase D.T.

5) Differentiation of *Gemella* spp., D. pigrum/R. Mucilaginosa (PYR+, LAP+)

	MAL	SUC	SOR	AlkP	ADH	Remarks
Gemella bergeriae	0	0	0	0	0	
Gemella haemolysans	+	V	0	+	0	
Gemella morbillorum	+	+	0^+	0	0	
Gemella sanguinis	$+^0$	+	+	+	0	
Dolosigranulum pigrum	+	+	0^+	0	$+^0$	
Rothia mucilaginosa	·	+	0	0	·	NO_3+, VP+

MAL = Maltose D.T., SUC = Sucrose D.T., SOR = Sorbitol D.T. and AlkP = Alkaline Phosphatase D.T.

6) Identification of *Candida glabrata* (9)

	TRE (4h)	SUC (4h)
Candida glabrata	+	0
Candida tropicalis	V	+
Other *Candida* spp.	0	V

TRE = Trehalose D.T., SUC = Sucrose D.T. Incubation 4 h at 37 °C, McF 2.

7) Very rapid (30-60 seconds) identification of *Candida glabrata* (10,11)

	TRE + Clinistix	SUC + Clinistix
Candida glabrata	+	0
Candida tropicalis	V	+
Other *Candida* spp.	0	V

TRE = Trehalose D.T., SUC = Sucrose D.T. The Diagnostic Tablets are crushed in saline (1 D.T. in 2 ml saline) and the supernatant used for testing.

8) Differentiation of *Bacillus* spp.

	PEN	ANA gr.	NO_3	MAN	ADH	LEC	MOT	Gelatinase	βhem
B. subtilis		0	+	+	0	0	+	·	
B. cereus	R	+	$+^0$	0	0^+	+	+	+	+
B. megaterium		0	0	+	0	0	+	·	
B. anthracis	S	+	+	0	0	+	0	0	0
B. thuringiensis (parasporal crystals in sporulated cultures)		+	+	0	+	+	+	+	·

ANA gr. = Anaerobic growth, LEC = Lecithinase, βhem=Beta haemolysis

9) Enterococcus/lactococcus

	SOR	ARA	42 °C
E. faecalis	+	0	+
E. faecium	V	+	+
Lact. garviae	0	0	0

42°C = growth at 42 °C.

10) Lactobacillus spp from human blood (Fosfo R)

Group pos, non-motile, non-sporeforming straight rods with rounded ends.

	ARA	RHAM	NO_3	Growth 20°C	Vanco	Cipro
L. acidophilus group	0	0	·	+	S	R
L. casei group						
L. casei	+	0	·	+	R	S
L. rhamnosus	+	+	·	+	R	S
L. plantarum	V	0	·	+	R	R
L. reuteri/fermentum group						
L. reuteri	+	0	0	0	R	I/R
L. fermentum	V	0	+	+	R	I/R

11) Differentiation of C. jeikeium, C. afermentans and C. coylae

	GLU	PYR	CAMP	Lipo	Res.
C.jeikeium	+	0	0	+	+
C. afermentans	0	0	$+^0$	V	0
C. coylae	(+)weak	76	+	0	0

Lipo=lipophilic, Res=Multiresistant

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Adonitol (Adonitol)	*K. pneumoniae* ATCC 13883	*E. coli* ATCC 25922
l-Arabinose (L-Arabinose)	*E. coli* ATCC 25922	*S. aureus* ATCC 25923
Glucose (D-Glucose monohydrate)	*P. aeruginosa* ATCC 27853	*A. lwoffii* ATCC 9957
Inulin (Inulin)	*S. bovis* ATCC 15351	*E. coli* ATCC 25922
Lactose (Lactose monohydrate)	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
Maltose (Maltose monohydrate)	*E. coli* ATCC 25922	*Morganella morganii* ATCC 25830
Mannitol (D-Mannitol)	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
Mannose	*E.coli ATCC 25922*	*Proteus vulgaris ATCC 13315*
Melibiose (D-Melibiose)	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
Raffinose (D-Raffinose pentahydrate)	*Enterobacter cloacae* ATCC 13047	*E. coli* ATCC 25922
l-Rhamnose (L-Rhamnose)	*K. pneumoniae* ATCC 13883	*Proteus vulgaris* ATCC 13315
Sorbitol (D-Sorbitol)	*E. coli* ATCC 25922	*Proteus vulgaris* ATCC 13315
Sucrose (Saccharose)	*Enterobacter cloacae* ATCC 13047	*Morganella morganii* ATCC 25830
Trehalose (D-Trehalose dihydrate)	*E. coli* ATCC 25922	*Morganella morganii* ATCC 25830
d-Xylose (d-Xylose)	*E. coli* ATCC 25922	*Morganella morganii* ATCC 25830

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3.37.0 TDA or INDOLE (TDA or IND)

REF No. 57811

Double test tablet that can be used for **either** the Indole test **or** the Tryptophan deaminase test (TDA).

The Tryptophan deaminase test differentiates **Proteus**, **Morganella**, and **Providencia** (positive reaction) from other Enterobacteriaceae (negative reaction) and thus replaces the Phenylalanine deaminase test.

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 **4 hours** or **18-24 hours**. The tablet can be used for **either** the Indole test **or** the Tryptophane deaminase test.

Reading of the tests

A) Indole

After incubation add 3 drops of Kovacs' reagent (92031), shake gently and wait for at least 3 minutes. Look only at the **color of the surface layer**.

Positive reaction: **Red** (purple, pink) (surface layer)
Negative reaction: Yellow

Results

1) The indole test is a well-known test used in the identification of Enterobacteriaceae and in the differentiation of anaerobes.

2) Actinobacillus/Pasteurella

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

3a) Differentiation of Propionibacteria (Metro I/R, Col R, Kana S, Vanco S, CAT+)

	IND	NO_3	ß-XYL	CAT
Propion acnes	+	$+^{0}$	0	+
Propion avidum	0	0	+	+
Propion granulosum	0	0	0	+
*Propion propionicum (*Arachnia*)*	0	+	0	0

3b) *Propionibacterium acnes*/Actinomyces (Metro I/R, Col R, Kana S, Vanco S)

	IND	NO_3	CAT
Prop. acnes	+	+	+
Actinomyces spp.	0	0	0 wk

IND = Indole D.T., URE = Urease D.T., NO_3 = Nitrate Reduction D.T., B-XYL = Beta-Xylosidase D.T., and CAT = Catalase.

4) Differentiation of Fusobacterium (BrG R, Kana 500 S, Col S, Fosfo S, Vanco 5 R, CAT 0, NO_3 0, yellow-green fluorescence)

	Microscopy	IND	PYR	ESC	Alk P	Rifa	ARG	Oxgall	Remarks
Fusobacterium mortiferum	pleomorphic	0	+	$+^0$	+	R (<16 mm)	+	R	
Fusobacterium necrophorum	pleomorphic	+	0	0	+	S^R	0	V	subsp. necrophorum LIP + subsp. funduliformis LIP 0
Fusobacterium nucleatum	fusiform (slender)	+	0^+	0	0	S (≥16 mm)	V	S	NO_3 0, PGUA 0
Fusobacterium varium		$+^0$	+	0	V	R	+	R	
Fusobacterium gonidiaformans	non fusiform	+	·	0	0	S		S	HIP +
Fusobacterium naviforme	boat shape	+	·	0	0	S		S	PGUA +
Fus. ulcerans		0						R	Clinda R, ONPG 0
Leptotrichia spp.	pleomorphic	0	$+^0$	+	+	·		V	BrG S

IND = Indole D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., ESC = Esculin Hydrolysis D.T., Alk P = Alkaline Phosphatase D.T., Rifa = Rifampicin Neo-S, Oxgall (S = zone, R = no zone). BrG = Brilliant Green D.T. (S ≥ 10 mm, R < 10 mm), Kana 500 = Kanamycin 500 µg (S ≥ 10 mm, R < 10 mm), Col = Colistin 10 Neo-S (S ≥ 10 mm, R < 10 mm), Fosfo = Fosfomycin Neo-S (S ≥ 20 mm), Vanco 5 = Vancomycin 5 µg Neo-S (S ≥ 20 mm, R ≤ 18 mm), ARG=Arginine Aminopeptidase D.T.

5) Identification of Fusobacterium, funduliformis/necrophorum (3)

(Metro S, Kana S, Pen S), β-hemolysis, chartreuse color (UV), IND+, AlkP+

	Microscopy	Colony	Colony margin	Erythrocyte agglut.
Fus. Necroph subsp funduliformis	Coccoid-pleomorphic curling rods	creamy	Entire	0
Fus. Necroph subsp necrophorum	Pleomorphic entire rods	waxy	Erose	+

B) Tryptophane deaminase (TDA)

After incubation add 2 drops of Ferric Chloride 10% solution. Read within 5 minutes.

Positive reaction: **Brown/red**
Negative reaction: Yellow/orange

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

Results

	TDA
Proteus spp.	+
Morganella morganii	+
Providencia spp.	+
Other Enterobacteriaceae	0

Quality Control

DIATABS (Active ingredients)		**Positive**	**Negative**
TDA or Indole: (L-Tryptophane)	**Indole**	*Proteus vulgaris* ATCC 13315	*K. pneumoniae* ATCC 13883
TDA or Indole: (L-Tryptophane)	**TDA**	*Proteus vulgaris* ATCC 13315	*K. pneumoniae* ATCC 13883

References

1) Funke G. et al: Clinical microbiology of Coryneform bacteria. Clin. Microbiol. Reviews **10**, 125-159, 1997.
2) Ashhurst-Smith C. et al: Actinobacillus equuli septicemia: an unusual zoonotic infection. J. Clin. Microbiol., **36**, 2789-90, 1998.
3) Jensen A. et al: Minimum requirements fro a rapid reliable routine identification and antibiogram of Fusobacterium necrophorum. Eur. J .Clin. Microbiol. Infect Dis. 27, 557-563, 2008.

3.38.0 TELLUR (TEL)

REF No. 45011

Contain potassium tellurite and are specially intended for the differentiation of ***Enterococcus faecalis*** from other enterococci and **streptococci**. The diffusible amount is 500 µg per tablet.

Procedure

Place one Tellur diagnostic tablet on an agar plate seeded with the culture to be tested and incubate overnight at 35-37 °C.

Enterococcus faecalis will normally grow on a tellurite containing substrate with black colonies, i.e. it will grow close to the edge of the Tellur diagnostic tablet showing a broad ring of black colonies, whereas other enterococci and streptococci will grow relatively far from the tablet.

Reading of the test

		TEL
E. faecalis:	**Grey/black colonies**, close to the edge of the tablet.	< 10 mm (R)
Streptococci and most other enterococci:		> 12 mm (S)

A few Tellur resistant strains are found among *E. casseliflavus*, *E. mundtii*, *E. faecium*, and *E. gallinarum*.

Quality Control

DIATABS (Active ingredients)	**Sensitive**	**Resistant**
Tellur 500 µg (Potassium tellurite)	*S. bovis* ATCC 15351	*E. faecalis* ATCC 29212

Reference

1) Facklam R.R.: Recognition of group D streptococcal species of human origin by biochemical and physiological tests. Appl. Microbiol. **23**, 1131-1139, 1972.

3.39.0 TETRATHIONATE REDUCTASE (TTR)

REF No. 57421

The enzyme tetrathionate reductase catalyzes the reduction of tetrathionate into thiosulphate. It has a diagnostic interest in the case of gram negative facultative anaerobes (Enterobacteriaceae, Vibrionaceae, etc.) and also in the case of gram negative strictly aerobes (Non-fermenting gram negative bacilli).

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 TTR Diagnostic Tablet and **3 drops of sterile paraffin oil**. Close the tube and incubate at 35-37 °C for **4 hours** or for 18-24 hours.

Reading of the test

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

The oil overlay provides anaerobic conditions necessary to avoid false positive reactions.

Results

1) Enterobacteriaceae

TTR positive	TTR negative
Edwardsiella	*E. coli*
Salmonella $+^0$	*Shigella* spp.
Citrobacter freundii	*Klebsiella* spp.
Serratia liquefaciens	*Enterobacter* spp.
Proteus spp.	*Serratia marcescens* (V)
Morganella	*Yersinia enterocolitica* (V)
Providencia spp.	

2) Non-fermenters

TTR positive	TTR negative
Comamonas acidovorans	*Ps. fluorescens*
Com testosteroni	*Ps. putida*
Shewanella putrefaciens	*Sphing. paucimobilis*
Shewanella algae	*Burkh. cepacia*
Sten. maltophilia	*Brev. diminuta*
Alcalig denitrificans $+^0$	*Ralst. pickettii*
Achr. xylosoxidans	*Acinetobacter* spp.
Past. multocida	

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Tetrathionate reductase (Tetrathionate)	*Proteus vulgaris* ATCC 13315	*E. coli* ATCC 25922

Reference

1) Richard C.: La tetrathionate-reductase (TTR) chez les bacilles a gram negatif: Interet diagnostique et epidemiologique. Bull. Inst. Pasteur, **75**, 369-382, 1977.
2) Freland C. et al: Campylobacter pyloridis: etude bacteriologique et sensibilite aux antibiotiques. Path. Biologie **35**, 1037-1042, 1987.
3) Le Minor L: Tetrathionate reductase, beta glucuronidase and ONPG-test in the genus Salmonella. Zentralbl. Bakteriol. (Orig A), **243**, 321-5, 1979.

3.40.0 TRIBUTYRIN (TRIB)

REF No. 48821

Test for enzymatic hydrolysis of tributyrin into butyric acid and glycerol. The release of butyric acid lowers pH and results in a color change from red to yellow. Mainly used in **differentiation of *Moraxella catarrhalis*** (positive within 4 hours) from *Neisseria* spp. (negative).

Procedure

Growth from an agar plate (oxidase positive, gram-negative diplococci) is suspended in 0.25 ml saline to achieve a turbidity corresponding to McFarland No. 4-5. Add one Tributyrin diagnostic tablet and close the tube. Incubate at 35-37 °C for **4 hours**. It is also possible to read after overnight incubation.

Reading of the test

Positive reaction: **Yellow**, yellow orange
Negative reaction: Red

Results

1) Moraxella/Neisseria

	TRIB
Moraxella catarrhalis	+
Neisseria spp.	0

2) Coryneform bacteria

	TRIB	PGUA
Coryn. glucuronolyticum	+	+
Coryn. renale	0	0

PGUA = Beta-Glucuronidase.

3) Differentiation of Moraxella/Psychrobacter (short, non-motile gram negative rods, OXI+, CAT+)

	TRIB	GLU	42 °C	URE	NO_3	Alk P	Remarks
M. catarrhalis	+	·	0	0	$+^0$	+	
M. nonliquefaciens	0	·	+	0	+	0	PRO +
M. lacunata	0	·	0	0	+	+	
M. osloensis	0	·	+	0	V	+	Yellow pigm., PYR 0
M. atlantar	0	0	0	0	0	+	PYR +, McConk + Growth stim. Bile, PRO 0, corroding
Ps. phenylpyruvicus	0	$+^0$	0	+	V	$+^0$	Growth stim. bile
Ps. immobilis	$+^0$	+	0	+	+	V	DEFRX S, growth not stim. bile
Ps. phenylpyruvicus-like	+	0	0	+	+	·	Growth stim. bile
Haemobacter spp.	+	0	0	+	0	+	

GLU = Glucose D.T., 42 °C = growth at 42 °C, URE = Urease D.T., NO_3 = Nitrate Reduction D.T., Alk P = Alkaline Fosfatase D.T., PRO = Proline Aminopeptidase D.T., McConk. = Growth in McConkey, Growth stim. bile = Growth stimulated by bile, DEFRX = Deferoxamine D.T. ($S \geq 16$ mm, $R \leq 14$ mm).

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Tributyrin (Tributyrin)	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922

References

1) Riou J.Y. et al: Hydrolyse de la tributyrine par les Neisseria et les Branhamella. (French). Ann.Microbiol. (Inst. Pasteur), **132A**, 159-169, 1981.
2) Riou J.Y., Guibourdenche: Branhamella catarrhalis. New methods of bacterial diagnosis. Drugs (suppl. 3), 1-6, 1986.
3) Christensen J.J. et al.: Branhamella catarrhalis: significance in pulmonary infections and bacteriological features. Acta path. microbiol. immunol scand, Section B, **94**, 89-95, 1986.
4) Richards J.: Evaluation of a rapid method for identifying Branhamella catarrhalis. J. Clin. Pathol. **41** ,462-464, 1988.
5) Cooke R.P.D.: Laboratory diagnosis of Branhamella catarrhalis. J. Clin. Pathol. **41**, 923, 1988.
6) Mannion P.T.: Tributyrin hydrolysis for identifying Branhamella catarrhalis. J. Clin. Pathol. **42**, 115, 1989.
7) Perez J.L. et al: Butyrate esterase (Tributyrin) spot test, a simple method for immediate identification of Moraxella catarrhalis. J. Clin. Microbiol. **28**, 2347-8, 1990.
8) 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.
9) Verduin C.M. et al: Moraxella catarrhalis: from emerging to established pathogen. Clin. Microbiol. Reviews **15**, 125-144, 2002.

3.41.0 UREASE (URE)

REF No. 57511

The hydrolysis of urea is catalyzed by a specific enzyme, urease, to yield two molecules of ammonia. In the presence of the indicator phenol red there is a change of color from yellow/orange to red/ purple in case of a positive reaction.

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 Urease diagnostic tablet, close the tube and incubate at 35-37 °C for **4 hours** or **18-24 hours**.

Reading of the test

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

After **overnight** incubation **only strong red or purple** should be considered as a positive reaction!

Results

1) Enterobacteriaceae

The rapidity by which there is a change of color (urease pos.) may have a diagnostic interest.

Morg. morganii show in most cases a **positive reaction** within 30 min.

The following species of Enterobacteriaceae usually show a **positive reaction** within 4 hours: *Proteus* spp., *Morg. morganii*, *Enterobact. gergoviae*.

Klebsiella pneumoniae/*Klebs. oxytoca* and *Yersinia* spp. show also **a positive reaction**, but in most cases after overnight incubation.

The remaining Enterobacteriaceae show a **negative reaction**.

2) Staphylococci

The following staphylococci usually show a **positive reaction**:
Staph. epidermidis, *Staph. hominis*, *Staph. warneri*, *Staph. simulans*, *Staph. saprophyticus*, *Staph. xylosus*, *Staph. aureus* (V), *S. lugdunensis* (V), *Staph. capitis* subsp. *ureolyticus*, *Staph*. *cohnii* subsp. *urealyticum*.

The following staphylococci show a **negative reaction**:
Staph. *capitis*, *Staph*. *haemolyticus*, *Staph*. *auricularis*, *Staph*. *schleiferi*, *Staph*. *cohnii*, *Staph*. *sciuri*, *Staph*. *lentus*.

3) Non-fermenters

The following non-fermenters usually show a **positive reaction**:
Flav. *odoratum*, *Ochrobactrum anthropi* (Group Vd), *Sph*. *multivorum*, CDC group IV c-2, *Oligella ureolytica* (IVe), *Bordetella bronchiseptica*, *Agrob*. *tumefaciens* (radiobacter).

4) Differentiation of Actinobacillus from Pasteurella

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

URE = Urease D.T., IND = Indole D.T.

5) Useful in identification of gram-negative anaerobes:
Bact. *ureolyticus* (+), *Bilophila* spp. (+0), *Desulfomonas pigra* (V). Others are negative.

6) Differentiation of lipophilic corynebacteria

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

	Res or multires.	URE	NO_3	PZA	HIP	GLU	SUC	αGLU	AlkP	Remarks
C. accolens		0	+	V	·	+	V	+	0	MAL 0
C. afermentans		0	0	+	0	0	0		$+^0$	LAP +, CAMP $+^0$
ssp. *lipophilicum*										
C. bovis		0^+	0	V	$+^0$	+	0		+	ONPG +
CDC group F-1		+	V	+	0	+	+		0	
C. pseudogenitalium										
CDC group G	+	0	V	+	V	+	V		+	FRU +
C. jeikeium (JK)	+	0	0	+	+	+	0	0	+	FRU 0, PRO 0, CAMP 0
C. macgingleyi		0	+	0	V	+	+	0	+	LAP 0,MAL0, conjunctive
C. urealyticum (D-2)	+	+	0	+	$+^0$	0	0		V	O/129 R^S, PRO 0
C. resistens	+	0	0	0	·	+	0	0	+	PYR
C. ureicelerivorans		$+^R$	0	+	+	+	0	0	+	PYR+, VP+, 0/129 R

URE = Urease D.T., NO_3 = Nitrate Reduction D.T., HIP = Hippurate Hydrolysis D.T., GLU = Glucose D.T., SUC = Sucrose D.T., AlkP = Alkaline Phosphatase D.T., αGLU = Alpha-Glucosidase S.D., LAP = Leucine Aminopeptidase D.T., PZA = Pyrazinamidase D.T., O/129 = O/129 D.T. (S ≥ 16 mm, R < 16 mm), Res = multiresistant, CAT = catalase, PRO = Proline Aminopeptidase, Fosfo = Fosfomycin Neo-S (R = no zone), Mupi = Mupirocin Neo-S (R = no zone), MOT = motility, PZA = Pyrazinamindase D.T., FRU = Fructose D.T.$+^R$=rapid positive.

7) Differentiation of *Brucella* spp. from similar organisms

All inoculation procedures and manipulation of possible cultures of *Brucella* spp. should be performed wearing gloves, in a biological safety cabinet.

	TRYP	PYR	CAT	OXI	URE	NO_3	MOT	Cell morphol.	Remarks
Brucella spp.	0	0	+	+	$+^R$	+	0	Tiny cbb stains faint	from blood, bone marrow, X or V factor not req.
Bordetella bronchiseptica	0	0	·	+	+	+	+	small ccb, rods	
Acinetobacter spp.	0	0	·	0	V	0	0	broad ccb	
Psychrobacter spp.			·	+	+	V	0	broad ccb	
Oligella ureolytica	0	0	·	+	$+^R$	+	V	tiny ccb	from urine
Pasteurella spp.			·	+wk	0^+	+	0	medium rods	IND +
Francisella tularensis			·	0	0	0	0	tiny ccb	
Haemophilus influenzae			·	V	V	+	0	small ccb	X or V factor req.
Haemobacter spp.			+	+	+	0	0	pleom. rods	TRIB+, LAP+, AlkP+
Ochrobactrum spp	+	+	+	+	V	+	·		Col R

OXI = Oxidase D.T., URE= Urease D.T., NO_3 = Nitrate Reduction D.T., MOT = motility, $+^R$ = rapid positive reaction, ccb = coccobacilli, IND = Indole D.T., wk = weak.

1) *Brucella* spp. appear as punctate smooth colonies, non pigmented, non haemolytic.
2) *Brucella* spp. do not grow on McConckey Agar.
3) *Brucella* spp. have a characteristic Gram stain morphology: tiny, faintly stained coccobacilli (differentiation from *Psychrobacter* spp.).
4) *Brucella* spp. are oxidase and urease positive (in most cases rapidly positive), and X or V Factors are not required for growth (differentiation from *haemophilus* spp.).
5) The most common misidentification of *Brucella* spp. is *Haemophilus influenzae* (requires X and V Factors) and *Psychrobacter* spp. (broad coccobacilli).

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Urease (Urea)	*Proteus vulgaris* ATCC 13315	*E. coli* ATCC 25922

References

1) Jousimies-Somer H.R. et al: Anaerobic gram-negative bacilli and cocci. Manual of Clinical Microbiology 5th Ed., 538-552, 1991.
2) Summanen P. et al: Wadsworth anaerobic Bacteriology Manual 5th Ed. Advanced Identification Methods (Level III) pages (49, 50, 65, 93, 159) 1993.
3) Ashhurst-Smith C. et al: Actinobacillus equuli septicemia: an unusual zoonotic infection. J. Clin. Microbiol. **36**, 2789-90, 1998.

3.42.0 VOGES-PROSKAUER (VP)

REF No. 57711

The Voges-Proskauer test is used in the differentiation among **Enterobacteriaceae** and in the **streptococci** group.

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 Voges-Proskauer Diagnostic Tablet and close the tube. Incubate at 35-37 °C for not more than **4 hours**.

Before reading, **add 2 drops of alpha-naphthol solution** (5% in ethanol) and afterwards 1 drop of 40% KOH and shake.

Reading of the test

Positive reaction: **Red/pink**
Negative reaction: Colourless (no change in colour) . Very light pink.

Wait 5 min before a test is considered negative.

Results

1) Enterobacteriaceae

	VP
Enterobacter spp., *Klebsiella pneumoniae/oxytoca*, *Serratia* spp., Hafnia	positive
Citrobacter spp., *E. coli*, *Klebsiella ozaenae/rhino-scleromatis*, *Morganella morganii*, *Proteus* spp. (except *Pr. mirabilis*: V), *Providencia* spp., *Salmonella* spp., *Shigella* spp.	negative

2) Yersinia

	VP(25°C)	SUC	PZA
Yersinia enterocolitica	+	$+^0$	0
Yersinia kristensenii	0	0	+

VP(25°C) = Voges Proskauer D.T. (at 25 °C), SUC = Sucrose D.T. and PZA = Pyrazinamidase D.T.

3a) Streptococci beta-haemolytic (human) (8) (Mupi S)

	VP(4h)	PYR	PGUA	SORB	HIP	CAMP	Colony Size	Remarks
S. pyogenes (Group A)	0	+	V	0	0	0	Large	MAN 0
S. anginosus group (Groups A, C, G, F)	+	0	0		0	0	Small	
Group B (*S. agalactiae*)	+	0	$+^0$	0	+	+	.	MAN 0
S .dysgalactiae subsp. *equisimilis* (ACG) *	0	0	+	0	0^w	0	Large	αGAL 0, TRE +,
S. iniae (no group)	0	+	+	0	0	+	Small	MAN +, AlkP+,ADH+, $NO_3$0
S. porcinus (E, P, U, V)	+	$+^0$	+	+	0^+	+		MAN +
S. pseudoporcinus	V	0^+	.	+	0	+	Small	MAN +,ADH+
S. canis (G)	0	V	0	0	0	+	Large	αGAL +, MAN 0
S. equi subsp equi (C)	0	0	+	0	0	.	Large	TREV, LACT0^+
S. equi subsp ruminatorum (C)	0	0	+	V	+	.	Large	LACT+, ESC 0
S. equi subsp. *zooepidemicus* (C)	0	0	+	+	0	0	Large	TRE 0, LACT+, ESC+
S. phocae (FG)								AlkP+, LAP+, ESC0
Arcanobact. haemolyticum	0	0	V		0	.		Mupi R

* may be alpha haemolytic sometimes.

VP(4h) = Voges Proskauer D.T. (4 h incubation), PYR = Pyrrolidonyl Aminopeptidase D.T., PGUA = Beta-Glucuronidase D.T., HIP = Hippurate Hydrolysis D.T., Mupi = Mupirocin Neo-S (S ≥ 16 mm, R < 16 mm), CAMP = CAMP test.

3b) Streptococci beta-haemolytic CAMP positive

	PYR	SORB	HIP
S. agalactiae	0	0	+
S. iniae	+	0	0
S. porcinus	+	+	0
S. pseudoporcinus	0	+	0

3c) Viridans streptococci (most current clinical isolates)

Most strains are: CAT 0, LAP +, PYR 0, BE 0.

A1) *S. mitis* group (VP 0)

	OPT	ADH	ONPG	αGAL	αFUC	TRE	ESC	Remarks
S. gordonii	R	+	+	V	+	+	+	PGUA 0, ß-MAN +, SUC +
S. parasanguinis	R	+	+	+^{0}	V	0^{+}	0	
S. sanguinis	R	+	0^{+}	V	0	+	+	AlkP 0, SUC +
S. mitis	R/S	0^{+}	+^{0}	+	0	0	0	(Oxgall R), AMYG +
S. oralis	R	0	+^{0}	0	0	V	0	Oxgall R, AMYG 0
S. suis	R	V	+	+	+	+	+	PGUA +^{0}, CAMP +, , MAN 0
S.sinensis	R	+	0	0	·	+	+	VP+,BE+
S. gallinaceus	R	+	+	+	+	+	+	PGUA 0, MAN +
S. massiliensis	R	+	0	0	0	0	0	HIP +, LAP +, AlkP +, MAL +, SUC 0
S. pneumoniae	S	+^{0}	+	+	0	+	V	Oxgall S
S. pseudopneumoniae	R/S	+	+	0	·	V	V	Oxgall R, TET R^{s}, ERY R^{S}

A2) *S. sanguinis* biotypes

	ESC	?-GAL	ONPG
S. sanguinis bio 1	+	V	0
S. sanguinis bio 2	+	+	V
S. sanguinis bio 3	0	0	+

B) *S. "milleri"*/anginosus group (ADH +, ESC +, MAN 0, PYR 0, VP +, SOR 0)

	NAG	ONPG	RAF	ß-FUC	ß-GLU	Remarks
S. anginosus	0	0	V	0	+	
S. constellatus	0	0	0	0	0	
S. constellatus subsp. *pharyngis*	+	+	0	+	+^{0}	
S. intermedius	+	+	0^{+}	+	V	
S. sinensis	0	0	0	·	+	BE+,αGAL0,AlkP0

C) *S. mutans* group (ADH 0, VP +, MAN +, SORB+)

	MEL	BaL	ß-GLU
S. mutans	+^{0}	R	+
S. sobrinus	0	R	0
S. cricetus	+	S (≥10 mm)	
S. downei(monkey)	0	S	
S. orisuis (swine)	+	S	+

D) *Bovis* group (ADH 0, most VP +, MAN 0)

	ONPG	α-GAL	VP	ESC	PGUA	TRE	MAN	Remarks
S. infantarius	0	+		0^{+}	0	0	0	MEL +
S. lutetiensis	0	$+^{0}$		+	0	0	0	MEL 0
S. gallolyticus (*bovis* I)	0	V	+	+	0	+	+	BE +
S. macedonicus	$+^{0}$	0^{+}	+	0	0	0	0	
S. pasteurianus	+	V	+	+	+	+	0	ß-MAN +
S. bovis (II)	0	$+^{0}$	0	+	0	V	0	BE +,VP 0

ADH = Arginine Dihydrolase D.T., α-GLU = Alpha-Glucosidase D.T., α-GAL = Alpha-Galactosidase D.T., α-FUC = Alpha-Fucosidase D.T., ESC = Esculin Hydrolysis D.T., NAG = Beta-N-Acetylglucosaminidase D.T., ß-FUC = Beta-Fucosidase D.T., ß-GLU = Beta-Glucosidase D.T., RAF = Raffinose D.T., BaL = Bacitracin Low D.T., VP = Voges Proskauer D.T. (4 hours incubation), Col. dry adh. = Colonies dry adherent. OPT = Optochin 10 µg D.T., TRE = Trehalose D.T., AMYG = Amygdalin.

4a) Coagulase positive staphylococci

	VP(4h)	PYR(1h)	Poly	PGUA	Col10	TRE
Staphylococcus aureus	+	0 wk	R (≤ 12 mm)	0	R(no zone)	
S. intermedius	0 wk	+	S (≥ 14 mm)	0	S	+
S. pseudintermedius	+	+	S (≥ 14 mm)	0	R	+
S. hyicus	0	0 wk	V	+	R	
S. schleiferi coagulans	+	+	S (≥ 14 mm)	0	S	0

VP(4h) = Voges Proskauer D.T. (4 h. incubation), PYR = Pyrrolidonyl Aminopeptidase D.T. (1 h. incubation), Poly = Polymyxins Neo-S, PGUA = Beta-Glucuronidase D.T.

4b) Differentiation of gram positive cocci from blood cultures (most common)

	PYR (1h)	αGAL	HIP	VP	Remarks
S. aureus	0	·	·	+	HCF +
St. pneumoniae	0	$+^{0}$	0	0	OPT S
Enterococci	+	V	V	+	BE+, GrD
St. group A	+	0^{+}	0	0	
St. group B	0	0	+	+	HCF 0

αGAL = Alpha-Galactosidase D.T., HIP Hippurate Hydrolysis D.T., VP = Voges Proskauer D.T., PYR = Pyrrolidonyl Aminopeptidase (1-hour incubation), HCF = Human clumping factor, OPT = Optochin D.T. (S ≥ 18 mm, R < 16 mm), BE = Bile Esculin D.T., GrD = Group D.

5) *Arcanobacterium* spp.

	VP(24h)	α-MAN	PYR	TRIB	XYL
Arcanob. pyogenes	+	0	82	0	+
Arcanob. haemolyticum	0	+	0	70	0

VP(24h) = Voges Proskauer D.T. (24h incubation), α-MAN = Alpha-Mannosidase D.T., PYR = Pyrrolidonyl Aminopeptidase D.T., TRIB = Tributyrin D.T., XYL = Xylose D.T.

6) Differentiation of Lactococcus spp

Most strains: BE+, LAP+, ADH+, Vanco S^{R}, non-motile, growth 10°C+

	PYR	VP	LACT	Clinda
L. lactis subsp lactis	V	+	+	S
L. lactis subsp cremoris	0	0	+	S
L. lactis subsp hordiae	0	0	0	S
L. garviae	+	+	+	R

Quality Control

DIATABS (Active ingredients)	**Positive**	**Negative**
Voges-Proskauer (Sodium Pyruvate 2 mg, Creatine)	*Enterobacter cloacae* ATCC 13047	*E. coli* ATCC 25922

References

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3) Mahoudeau I. et al: Frequency of isolation of Staph. intermedius from humans. J. Clin. Microbiol. **35**, 2153-4, 1997.
4) Guiyoule A. et al: Phenotypic and genotypic characterization of virulent Yersinia enterocolitica strains unable to ferment sucrose. J. Clin. Microbiol. **36**, 2732-4, 1998.
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7) Ruoff K. et al: Streptococcus in Manual of Clinical Microbiology. 7th ed. **17**, 283-296, 1999.
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4.1.0 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, 59121, 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.

5.1.0 Useful TABLES for bacterial identification / differentiation

1) Enterobacteriaceae
2) Non-Fermenters
3) Vibrio / Aeromonas / Plesiomonas
4) Staphylococci / Micrococci / Kitococcus
5) Enterococci
6) Streptococci / Pneumococci
7) Catalase Negative, Gram Positive Cocci
8) Pediococcus / Leuconostoc / Enterococcus / Weisella / Lactobacillus
9) Arcanobacterium
10) Neisseria / Moraxella / Psychrobacter / Brucella
11) Haemophilus / HACEK Group / Aggregatibacter
12) Corynebacteria
13) Gardnerella / Mobiluncus / Atopobium
14) Actinobacillus / Pasteurella
15) Actinomyces / Propionibacterium
16) Campylobacter / Helicobacter
17) Bacillus
18) Anaerobes
19) Nocardia
20) Yeast
21) Legionella

Doc. no.

1) ENTEROBACTERIACEAE

2) NON-FERMENTERS

Doc. no.

3) VIBRIO / AEROMONAS / PLESIOMONAS

4) STAPHYLOCOCCI / MICROCOCCI / KITOCOCCUS

5) ENTEROCOCCI/LACTOCOCCI

6) STREPTOCOCCI / PNEUMOCOCCI

7) CATALASE NEGATIVE, GRAM POSITIVE COCCI

8) PEDIOCOCCUS / LEUCONOSTOC / WEISSELLA/ Lactobacillus

9) ARCANOBACTERIUM

10) NEISSERIA / MORAXELLA / PSYCHROBACTER / BRUCELLA

11) HAEMOPHILUS / HACEK GROUP /AGGREGATIBACTER

12) CORYNEBACTERIA

13) GARDNERELLA / MOBILUNCUS/ ATOPOBIUM

14) ACTINOBACILLUS / PASTEURELLA

15) ACTINOMYCES/ Propionibacterium

16) CAMPYLOBACTER / HELICOBACTER

Doc. no.

17) BACILLUS

18) ANAEROBES

19) NOCARDIA

20) YEAST

21) LEGIONELLA

6.1.0 Alphabetic INDEX of Abbreviations and Codes

A) ACM = Acetamide Hydrolysis Diatabs (55721)
ADH = Arginine Dihydrolase Diatabs (56211)
ADO = Adonitol Diatabs (52011)
AER = Aerotolerant strains
ALA (dALA) = Porphyrin (d-Ala) Diatabs (57321)
Alk P = Alkaline Phosphatase Diatabs (55921)
ARA = l-Arabinose Diatabs (52121)
ANAgr = Anaerobic growth
AMP (AMP33) = Ampicillin 33 µg Neo-Sensitabs (70412)
AmpC = AmpC beta-lactamases
ARG=Arginine Aminopeptidase Diatabs (10061)

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) = Boronic Acid 250 µg Diatabs (10041)
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 (10031)
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 (59921)
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 (50021)
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 (59121)
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 (57821)
TEL = Tellur 500 µg Diatabs (45011)
TRE = Trehalose Diatabs (53911)
TRIB = Tributyrin Diatabs (48821)
TRYP = Trypsin Diatabs (47211)
TTR = Tetrathionate Reductase Diatabs (57421)

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 (54021)
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.