Identification of Enterococci

Enterococci are Gram-positive cocci in chains. They grow in 6.5 % NaCl and at 45 °C. Enterococci colonies are usually alpha- or gamma-haemolytic.

A NEW, easy and economical set-up using only two Neo-Sensitabs and two Diatabs for the identification of the most common enterococci of clinical importance

Introduction

Enterococci are common causes of hospital-acquired infections and vancomycin-resistant *E. faecalis* and *E. faecium* outbreaks are described with increasing frequency.
Identification of the Enterococcus species therefore becomes more important, especially the difficult differentiation between vancomycin-resistant *E. faecium* and the intrinsic low-level glycopeptide resistant strains, *E. gallinarum* and *E. casseliflavus.*

Since the resistance genes are transferable and non-transferable, differentiation of species is important if transmission has to be prevented.
Table I can be used for identification of isolates of the most clinically relevant Enterococcus.

The difficulties in identification of enterococcal species may be complicated by atypical or clinical uncommon strains and further testing may be necessary – ***Table II.***

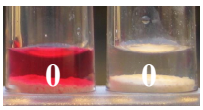
Table I. Identification of Most Clinically Relevant Enterococci*

	FURAZ	MUPIR	MOT	PIGM[1]	XYL (rapid)	α-GAL	Remarks
E. faecalis	**S**	**R**	0	0	**0**	**0**	TEL R
E. faecium	**R**	**S**	0	0	**0**	V	Clin R
E. gallinarum	S	S	+	0	**+**	**+**	HIP +[0]
E. casseliflavus	S	S	+	+	V	**+**	HIP 0
E. durans	S	S	0	0	**0**	**0**	Clin S

(1) Other pigmented enterococci: *E. flavescens, E. mundtii, E. sulfureus, E. gilvus, E. pallens* (MOT 0)

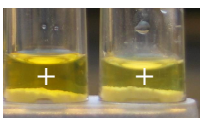
FURAZ = Furazolidone 50 µg Neo-Sensitabs, MUPIR = Mupirocin 10 µg Neo-Sensitabs, MOT = Motility, PIGM = Pigment production, XYL (rapid) = d-Xylose Diatabs used as described below, α-GAL = Alpha-Galactosidase Diatabs, TEL = Tellur Diatabs, (R = Resistance, i.e. growth to the edge of the tablet and black/grey colonies), HIP = Hippurate Hydrolysis Diatabs, Clin = Clindamycin 2 µg Neo-Sensitabs.

XYL α-GAL



E. faecalis

XYL α-GAL



E. gallinarum

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Procedure

	FURAZ	MUPIR	α-GAL	XYL Rapid xylose fermentation test
Media/Suspension	Mueller-Hinton Agar McFarland 0.5	Mueller-Hinton Agar McFarland 0.5	McFarland 4 in 0.25 ml saline. Add diagnostic tablet and close tube.	McFarland 4 in 0.25 ml saline. Add diagnostic tablet and close tube.
Incubation	Overnight at 35-37 °C	Overnight at 35-37 °C	4 hours at 35–37 °C	**2 hours** in a 37 °C water bath
Results	S ≥ 16 mm R: No zone	S ≥ 16 mm R: No zone	Positive: Yellow Negative: Colourless	Positive: Yellow,Yellow-Orange Negative: **Red, orange-red**

Table II. Identification of Enterococci*

	ADH	HIP	α-GAL	MAN	SOR	ARA	RAF	TEL	MOT	Remarks
E. avium	0	0^+	0	+	$+^0$	+	0	S	0	
E. raffinosus	0	V	+	+	+	$+^0$	+	S	0	
E. malodoratus	0	V	+	+	$+^0$	0	+	S	0	VP 0
E. pseudoavium	0	V	0^+	+	+	0	0	S	0	
E gilvus	0	0	+	+	+	0	+	S	0	Pigm +, PYR +
E. pallens	0	+	+	+	+	+	+	S	0	Pigm +, PYR 0
E. hermanniensis	0	0	0	+	0	0	0	S	0	VP +, LAC 0
E. saccharolyticus	0	0	+	+	+	0	+	S	0	PYR 0, Pigm 0
E. faecalis	+	V	0	+	+	0	0	R	0	Clin R
E. gallinarum	+	$+^0$	+	$+^0$	V	+	+	V	+	Pigm 0, Clin S
E. faecium	+	82	0^+	$+^0$	V	+	0^+	S^R	0	Clin V
E. casseliflavus	$+^0$	0	+	$+^0$	40	+	+	V	+	Pigm +, Clin S
E. mundtii	+	0	$+^0$	+	V	+	$+^0$	V	0	Pigm +
E. durans	+	V	0	0	0	0	0^+	S	0	SUC 0, Clin S
E. dispar	+	V	+	0	0	0	V	S	0	
E. hirae	+	V	$+^0$	0	0	0	V	S	0	SUC 88
E. sulfureus	0	0	+	0	0	0	+	S	0	Pigm +
E. cecorum	0	0	$+^0$	0	0	0	+	S	0	PYR 0, PGUA $+^0$, SUC +, VP +

ADH = Arginine Dihydrolase Diatabs, HIP = Hippurate Hydrolysis Diatabs, α-GAL = Alpha-Galactosidase Diatabs, MAN = Mannitol Diatabs, SORB, = Sorbitol Diatabs, ARA = Arabinose Diatabs, RAF = Raffinose Diatabs, TEL = Tellur Diatabs (R = Resistance, i.e. growth to the edge of the tablet and black/grey colonies), MOT = Motility, VP = Voges-Proskauer Diatabs, Clin = Clindamycin 2 µg Neo-Sensitabs, Pigm = Pigment production, LAC = Lactose Diatabs, SUC = Sucrose Diatabs, PYR = Pyrrolidonyl Aminopeptidase Diatabs, PGUA = Beta-Glucuronidase Diatabs.
+ = > 90 % positive strains, $+^0$ = 75 - 90 % positive, V = 26 –74 % positive strains, 0^+ = 10 - 25 % positive and 0 = less than 10 % positive strain

*Group D antigen, Bile-Esculin positive, PYR positive, Vancomycin (most S).

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	Neo-Sensitabs	Diatabs	Remarks
Table I	74412 Furazolidone 50 µg 75712 Mupirocin 10 µg	50211 Alpha-Galactosidase (α-GAL) 54021 d-Xylose (XYL)	**Neo-Sensitabs** 73612 Clindamycin 25 µg
Table II		50211 Alpha-Galactosidase (α-GAL) 56211 Arginine Dihydrolase (ADH) 56711 Hippurate Hydrolysis (HIP) 52121 l-Arabinose (ARA) 53011 Mannitol (MAN) 53311 Raffinose (RAF) 53711 Sorbitol (SOR) 45011 Tellur (TEL)	**Diatabs** 59011 Beta-Glucuronidase (PGUA) 52811 Lactose (LAC) 47011 Pyrrolidonyl Aminopeptidase (PYR) 53811 Sucrose (SUC) 57711 Voges-Proskauer (VP)

More information about the individual product is available in the User's Guides for Diatabs and Neo-Sensitabs on the website www.rosco.dk.

References:
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2. Tyrrell G.J. et al: *E. gilvus* sp. nov. and *E. pallens* sp. nov. isolated from human clinical specimens. J. Clin. Microbiol. **40**, 1140-5, 2002.
3. Teixeira L.M. et Facklam R.R. Enterococcus. IN: Murray P.R., Baron E.J., Jorgensen J.H. Pfaller M.A., Yolken R.H., editors. Manual of Clinical Microbiology 8th ed. Washington D.C.: American Society for Microbiology 2003, pp.422-433.
4. Chen D.K. et al: Evaluation of D-xylose and 1% Methyl-ß-D-Glucopyranoside fermentation tests for distinguishing *Enterococcus gallinarum* from *Enterococcus faecium*. J. Clin. Microbiol. **38**, 3652-5, 2000.

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