Molecular Detection of Vancomycin Resistant Enterococci

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VRE

- Members of the genus *Enterococcus* are:

Gram positive Cocci
Catalase : negative
Occur singly or arranged in pairs or short chains
Facultative anaerobes
May be β haemolytic (approximately 30%)
Hydrolyse esculin in the presence of bile salts.

VRE

The enterococci are commensal organisms

Act as opportunistic pathogens particularly in:

- Elderly with serious underlying disease
- Immunocompromised patients with long hospitalisation
- Treated with invasive devices and/or been treated with a broad spectrum antimicrobial.

VRE

- *E. faecalis* is the most common enterocccal species isolated.
- *E. faecium* is the second most common.
- Others:
- *E. gallinarum, E. avium, E. dispar,*

VRE

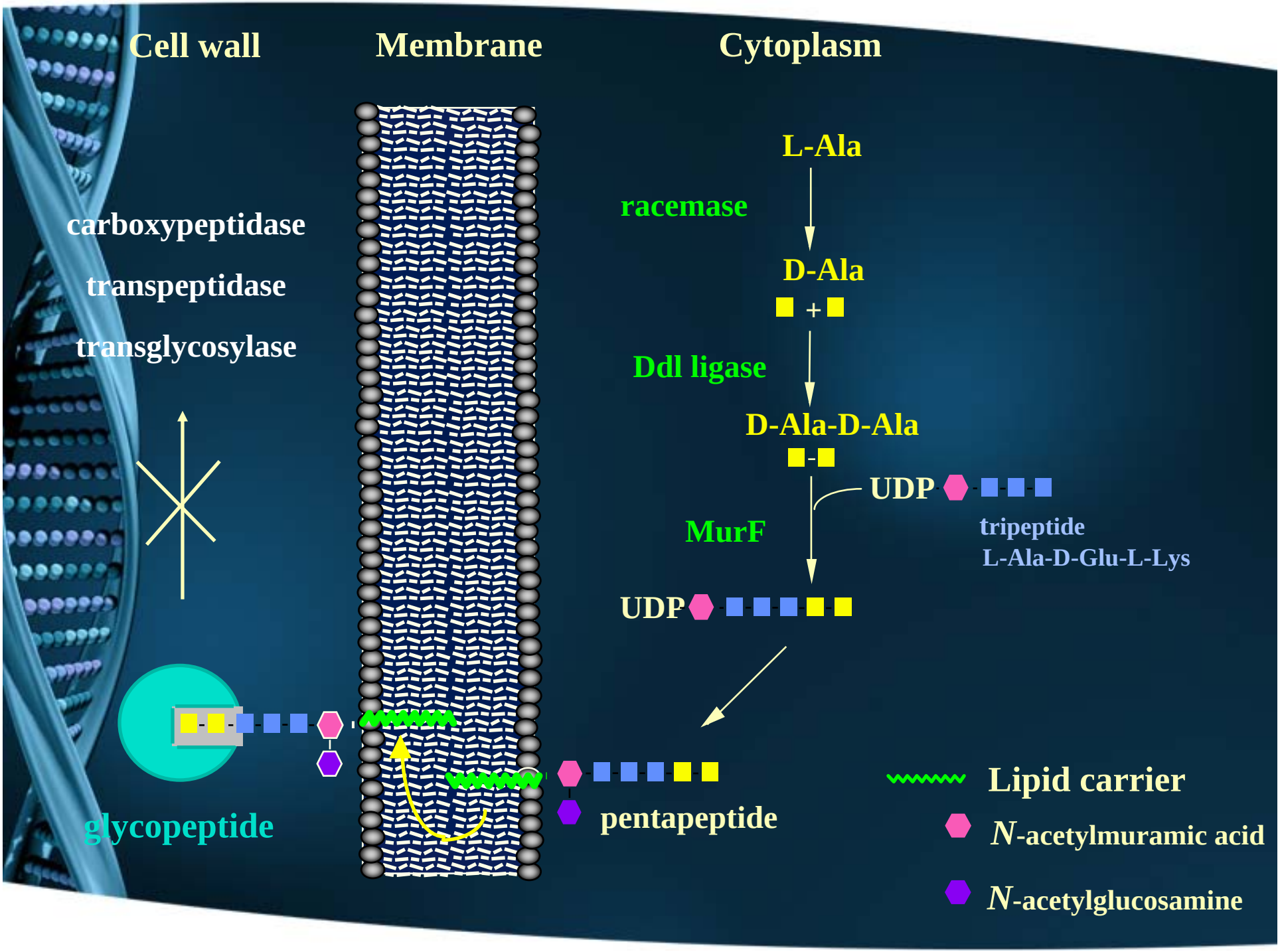
- Isolation of enterococci can be performed on any blood agar based medium
- They grow well at 35°C – 37°C and do not require a $C0_2$ environment.

Vancomycin

- Vancomycin is a complex glycopeptide antibiotic that was isolated in 1956 from a soil sample containing a newly discovered actinomycete, *Nocardia orientalis*.
- Large glycopeptide with a molecular weight of 1,449 daltons (larger than any penicillin, cephalosporin, tetracycline, aminoglycoside or macrolide).
- Vancomycin is taken orally or intravenously.

Vancomycin

- Inhibits synthesis of intact peptidoglycan (required for bacterial cell walls)
- Weakened cell wall - the cell undergoes lysis
- Glycopeptides bind to peptidyl-D-alanyl-D-alanine termini of peptidoglycan precursors and prevents transglycosylation and transpeptidation steps of cell wall peptidoglycan synthesis


Cell wall
Membrane
Cytoplasm
carboxypeptidase
transpeptidase
transglycosylase
L-Ala
racemase
D-Ala
+
Ddl ligase
D-Ala-D-Ala
UDP
tripeptide
L-Ala-D-Glu-L-Lys
MurF
UDP
glycopeptide
pentapeptide
Lipid carrier
N-acetylmuramic acid
N-acetylglucosamine

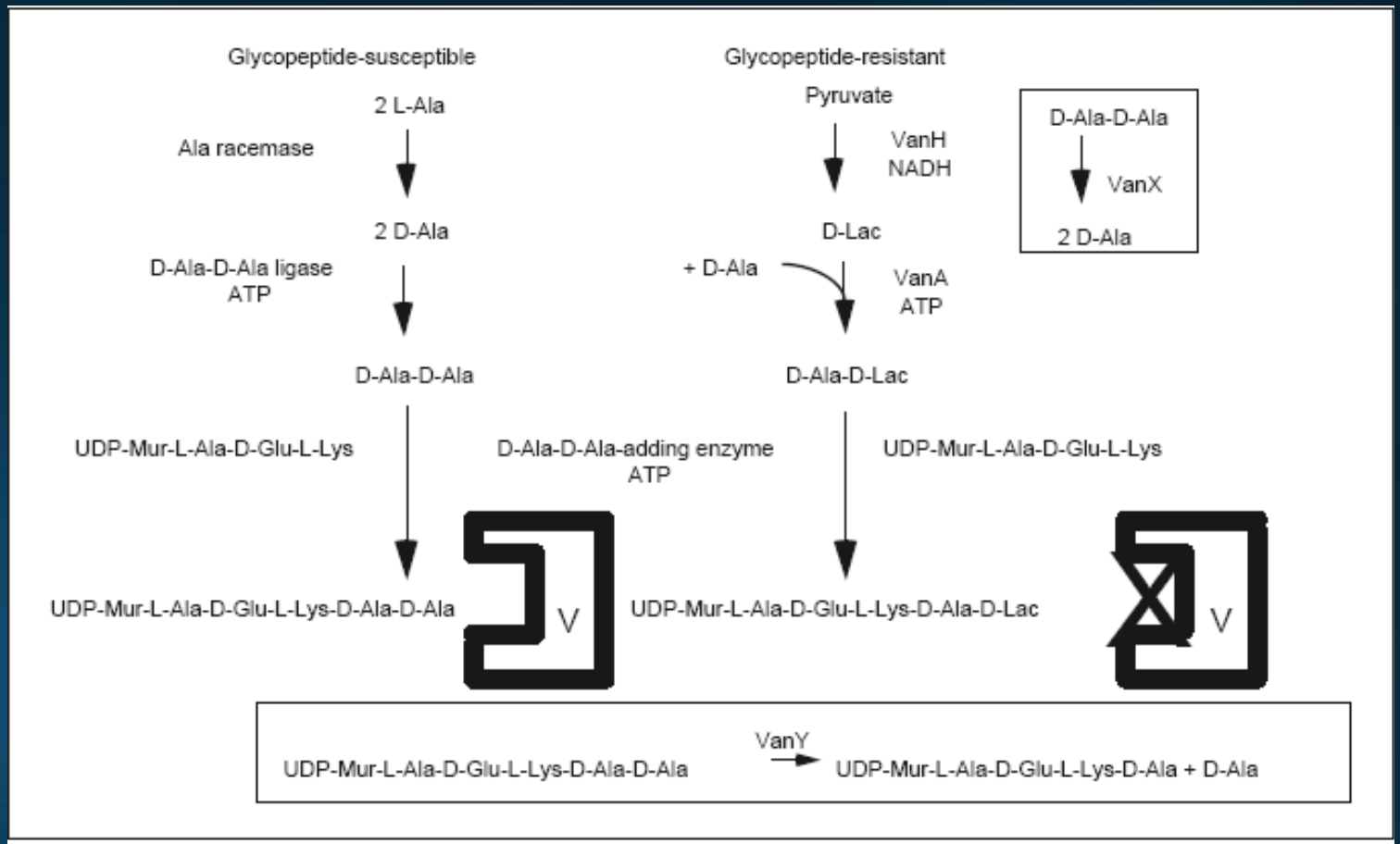


FIGURE 1. Mechanism of action of vancomycin and mechanisms of vancomycin resistance in enterococci with *vanA*-associated vancomycin resistance. V = vancomycin. From Patel R. Vancomycin-resistant enterococci in solid organ transplantation. *Curr Opin Organ Transplant*. 1999;4:271-280, with permission from Lippincott Williams & Wilkins.

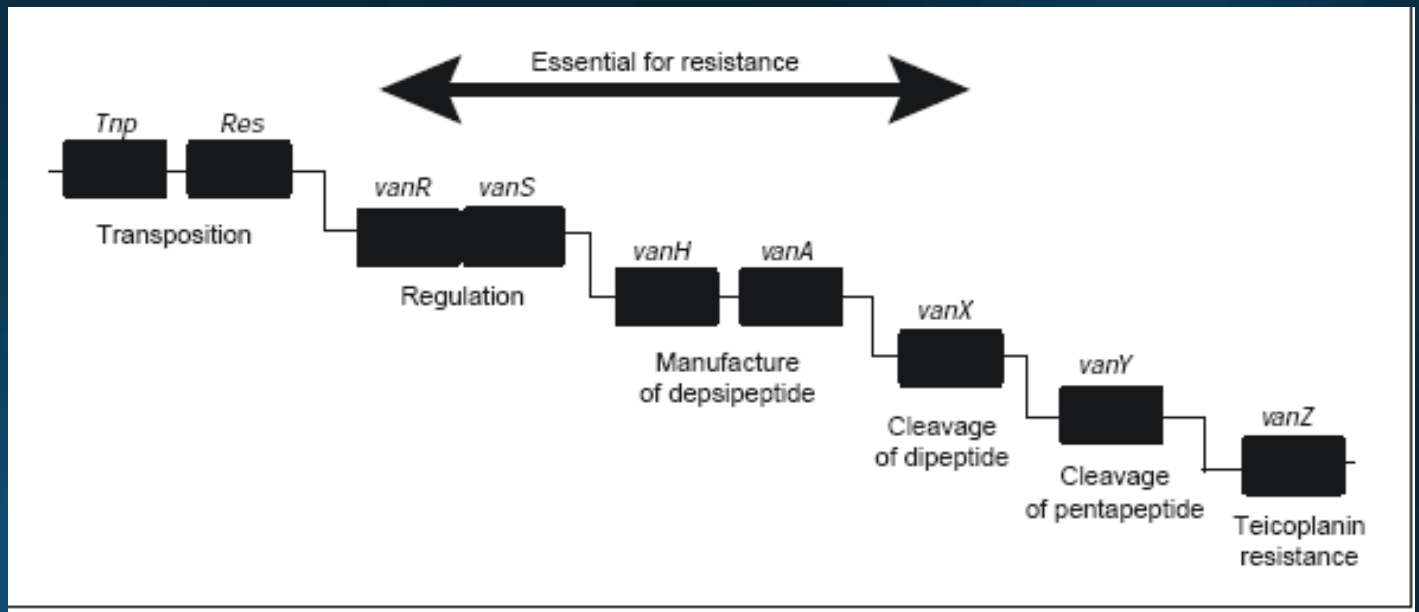


FIGURE 2. The *vanA* operon in Tn*1546*. From Patel R. Vancomycin-resistant enterococci in solid organ transplantation. *Curr Opin Organ Transplant*. 1999;4:271-280, with permission from Lippincott Williams & Wilkins.

Glycopeptide Resistance in Enterococci

Resistance	Acquired					Intrinsic
Phenotype	VanA	VanB	VanD	VanE	VanG	VanC
MIC (mg/L)						
Vancomycin	64 - >1,000	4 - 1,024	64 - 128	8-32	16	2 - 32
Teicoplanin	16 - 512	0.5 - 1	4 - 64	0.5	0.5	0.5 - 1
Expression	Inducible	Inducible	Constitutive	Inducible	Constitutive	Constitutive/ Inducible
Location	Plasmid Chromosome	Plasmid Chromosome	Chromosome	Chromosome		Chromosome
Transferable	Yes	Yes	No	No	No	No
Most Frequent enterococcal spp	*faecium & faecalis*					*gallinarum casseliflavus flavescens*

High Rate of False-Negative Results of the Rectal Swab Culture Method in Detection of Gastrointestinal Colonisation with Vancomycin Resistant Enterococci

D'Agata et al CID 2002

	Rectal swab culture		
VRE stool density $\log_{10}$ cfu/g stool	samples (n)	VRE detected	Sensitivity %
≥ 2.5	26	15	58
≥ 3.5	19	15	79
≥ 4.5	18	15	83
≥ 5.5	15	14	93
≥ 6.5	13	12	92
≥ 7.5	6	6	100

VANCOMYCIN RESISTANT ENTEROCOCCI (VRE) POLICY

“A major reservoir for VRE is unrecognized colonised patients in hospitals and proximity to a colonised patient is a major risk factor for acquisition. VRE can cause septicaemia in patients with those most at risk being high-risk surgery patients, patients who are immunocompromised and those undergoing dialysis. Transmission of VRE is primarily directly via contaminated hands and clothing of health care workers and indirectly via contaminated equipment and or environment”.

Surveillance Screening of High Risk Wards

- Patients who are direct transfers or have been in a hospital or residential care facility outside WA
- Any patient who has been in a ward with an unisolated VRE carrier during an outbreak
- Any patient who has had VRE isolated on any site
- Intensive Care Unit (ICU)
- High Dependency Area
- Bone Marrow Transplant Unit
- Nephrology Unit
- Haemodialysis Units - monthly
- Geriatric Ward

Enterococcosel Vancomycin Broth (EVB)

- EVB is a selective medium for the cultivation and differentiation of enterococci.
- The broth contains 8mg/L of Vancomycin, esculin and ferric ammonium citrate.
- Any enterococci will hydrolyse the esculin to produce esculetin which in turn reacts with ferric ammonium citrate to form a dark brown or black complex.
- Oxgall (source of bile) and sodium azide are also added which inhibit gram positive and gram negative organisms respectively.

EVB Broths

- Rectal swapped snapped into EVB
- Incubate 35°C for 24 hours for patient
- Incubate 35°C for 36 hours environment swabs
- EVB no colour change – negative
- EVB with a colour change (black) for PCR.

VRE Surveillance Screening
Royal Perth Hospital

Enterococcosel™ Broth
Incubate 24hrs 35ºC

Negative : **VRE not Isolated**
(approximately 50% of broths)

Positive : **PCR for vanA and vanB**

CULTURE

- EVB positive by PCR
- Subculture

Chromagar with 4mg/L Vancomycin

Chromagar

Blood agar

CHROMagar Microbiology

CHROMagar™ Orientation

- Chromogenic media allows the differentiation of *Enterococcus species* (blue colonies) from other enteric bacteria
  - *E. coli* (red colony)
  - *Klebsiella sp* (steel blue colony)
  - *Proteus* (brown halo colony)
- 24 hours incubation
- Contains the chromogenic substrate x-Gluc which targets β-glucosidase and allows the specific detection of enterococci

24hrs

CULTURE

- Culture
- Dark blue colonies subcultured onto BA for PCR confirmation.

VRE Surveillance Screening
Royal Perth Hospital

CLSI Kirby Bauer Disc Diffusion
Incubate and examine 24 hrs at 35ºC

Vancomycin 30μg disc

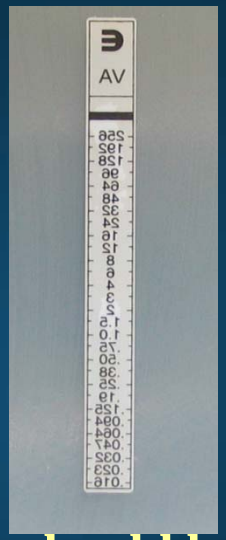
Resistant	**≤14mm zone diameter**
Intermediate	**15-16mm zone diameter**
Sensitive	**≥17mm zone diameter**

"Plates should be held for a full 24 hours and examined using transmitted light. The presence of a haze or any growth within the zone of inhibition indicates resistance. Organisms with an intermediate zone should be tested by an MIC method"

72hrs

VRE Surveillance Screening
Royal Perth Hospital

Vancomycin Etest
Incubate 24hrs 35°C



Vancomycin

Resistant	**≥ 32 mg/L**
Intermediate	**8-16 mg/L**
Sensitive	**≤ 4 mg/L**

"Plates should be held for a full 24 hours for accurate detection of resistance. For isolates with vancomyvin MICs of 8 - 16 mg/L perform biochemical tests for identification"

96hrs

vanB Enterococcus faecium Outbreak
Royal Perth Hospital

Period of the Outbreak

July 2001 - December 2001

Index case

July 2001 (Blood Cultures - ICU)

Number of VRE Cases

169 colonised

4 infections

Number of Wards Involved

23 (2 campuses)

vanB Enterococcus faecium Outbreak
Royal Perth Hospital

vanB Enterococcus faecium

Antibiogram

amoxycillin resistant
vancomycin resistant (MIC > 256mg/L)

teicoplanin sensitive (MIC 1.0mg/L)
gentamicin (high level) susceptible

Molecular Characteristics

PCR : *vanB* gene detected
PFGE : distinct CHEF pattern (8 subtypes)
Plasmid profile : distinct and consistent

vanB Enterococcus faecium Outbreak
Royal Perth Hospital
Patient and Environmental Screening

Total number of patient screening specimens	**19,658**
Total number environmental specimens	**24,396**
Total number positive patients	**169**

vanB Enterococcus faecium Outbreak Royal Perth Hospital

- Direct Culture

Rectal Swabs initially inoculated onto CHROMagar supplemented with 6mg/L vancomycin

VRE Surveillance Screening
Royal Perth Hospital

Direct Detection - Enrichment Broth

- ? VRE
  - Subculture on BA, Chrom
  - ? Enterococcus species
    - PCR
    - Biochemical Identification
    - Vancomycin and teicoplanin Etest & disk diffusion
    - PFGE

Real-Time PCR for the Rapid Detection of *vanA* and *vanB* Genes

Palladino et al *Diagn Microbiol Infect Dis 2002*

Roche Light Cylcer

- **Rapid Real time PCR in a closed system**

Simultaneous detection of *vanA* and *vanB genes*

Hybridisation probes purpose designed using the Roche Applied Science LightCycler Probe Design Software

Reduced potential for amplicon contamination

vanB Enterococcus faecium Outbreak
Royal Perth Hospital

- 1 *E. durans vanA*
- 4 *E dispar vanA* (4 PFGE types)
- 5 *E. faecalis vanE* (5 PFGE types)
- 10 *E faecium vanA* (7 PFGE)
- 126 *E faecium vanB* (3 PFGE types)
- 1 *E hirae vanA*
- 1 *E species vanA*

vanB Enterococcus faecium Outbreak
Royal Perth Hospital

Roche LightCycler

- **Results available within 1.5hrs**
- **Significant labour savings were realised**
- **Potential for direct specimen testing**

vanB Enterococcus faecium Outbreak
Royal Perth Hospital

EVB

- **50% required subculturing**
- **38% of subcultured broths grew colonies resembling enterococci**
- **Very labour intensive**

vanB *Enterococcus faecium* Outbreak
Royal Perth Hospital

Rapid Detection of *VanA* and *VanB* Genes Directly from Clinical Specimens and Enrichment Broths by Real Time Multiplex PCR

***Palladino et al J Clin Microbiol* JCM 2003**

Roche Light Cycler

Qiagen QIAamp DNA Stool Mini Kit

Total positive by PCR or Culture : 100 (known positive VRE patients)

	Rectal Swab	Enrichment Broth
Culture	43	75
PCR	45	88

vanB *Enterococcus faecium* Outbreak
Royal Perth Hospital

Limitations

- Van gene PCR-positive/culture negative

  - low number of cfu of VRE /gm faeces
  - non viable VRE
  - van gene detected in other genera
    - Anaerobic clostridia
    - *Bacillus circulans*
    - *Bacillus popilliae*
    - *Oerskovia lurbata*
    - *Arcanobacterium (Corynebacterium) haemolyticum*
    - *Staphylococcus aureus*

vanB *Enterococcus faecium* Outbreak
Royal Perth Hospital

Advantages

- **Greater sensitivity**
  - enrichment broth culture 75%
  - enrichment broth PCR 88%

- **Significant labour cost savings**
  - enrichment broth culture $15.41/sample ($8.98 labour)
  - enrichment broth PCR $12.93/sample ($3.37 labour)

- **Earlier clearance of VRE negative swabs**
  - 99% within 28 hours

- **Earlier detection of VRE**
  - (48 - 72 hours)

Current VRE Detection

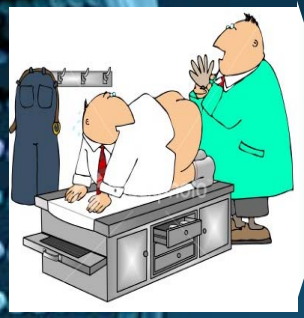
Front End Robotics

Currently using Roche MagNA Pure Instrument (reduces RSI and hands on time)

Validating on the LightCycler 480 for higher throughput.

BD GeneOhm™ VanR Assay

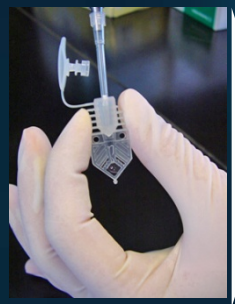
Test Procedure Overview



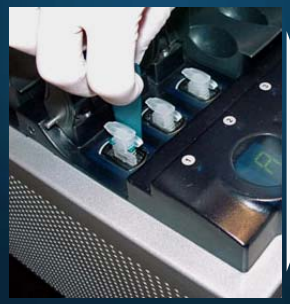
Perianal or Rectal swab

Specimen Prep

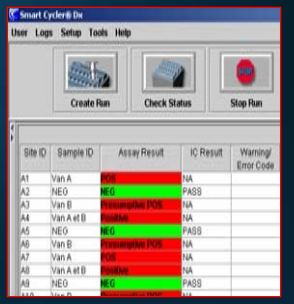
Lysis - DNA Extraction



Reconstitution



Real-time PCR Analysis on the SmartCycler® Instrument



Definitive On-screen Results

Results in < 2 hours

Results Interpretation

Sample Type	Instrument-Reported Assay Result	Instrument-Reported IC Result	User Interpretation of Results
Clinical Specimen	NEG	PASS	No *vanA or vanB* detected
	POS	NA	DNA from *vanA* resistance gene detected (no *vanB* detected); probable vancomycin resistant enterococci (VRE).
	Presumptive POS	NA	DNA from *vanB* resistance gene detected (no *vanA* detected); presumptive vancomycin resistant enterococci (VRE), *vanB* resistance gene may be from another organism.
	Positive	NA	DNA from *vanA* and *vanB* resistance genes detected; probable vancomycin resistant enterococci (VRE).
	Unresolved	FAIL	Unresolved; inhibitory specimen or reagent failure.
	ND	ND	Not determined due to I-CORE® Module failure (with Warning or Error Code[1]).

vanA detected - Probable VRE
vanA and *vanB* detected - Probable VRE
vanB only detected - May be VRE or resistance gene may be from another organism

1. BD survey, vanB rates.xls

Results with BD vanR Assay Compared to Culture

VanR Assay

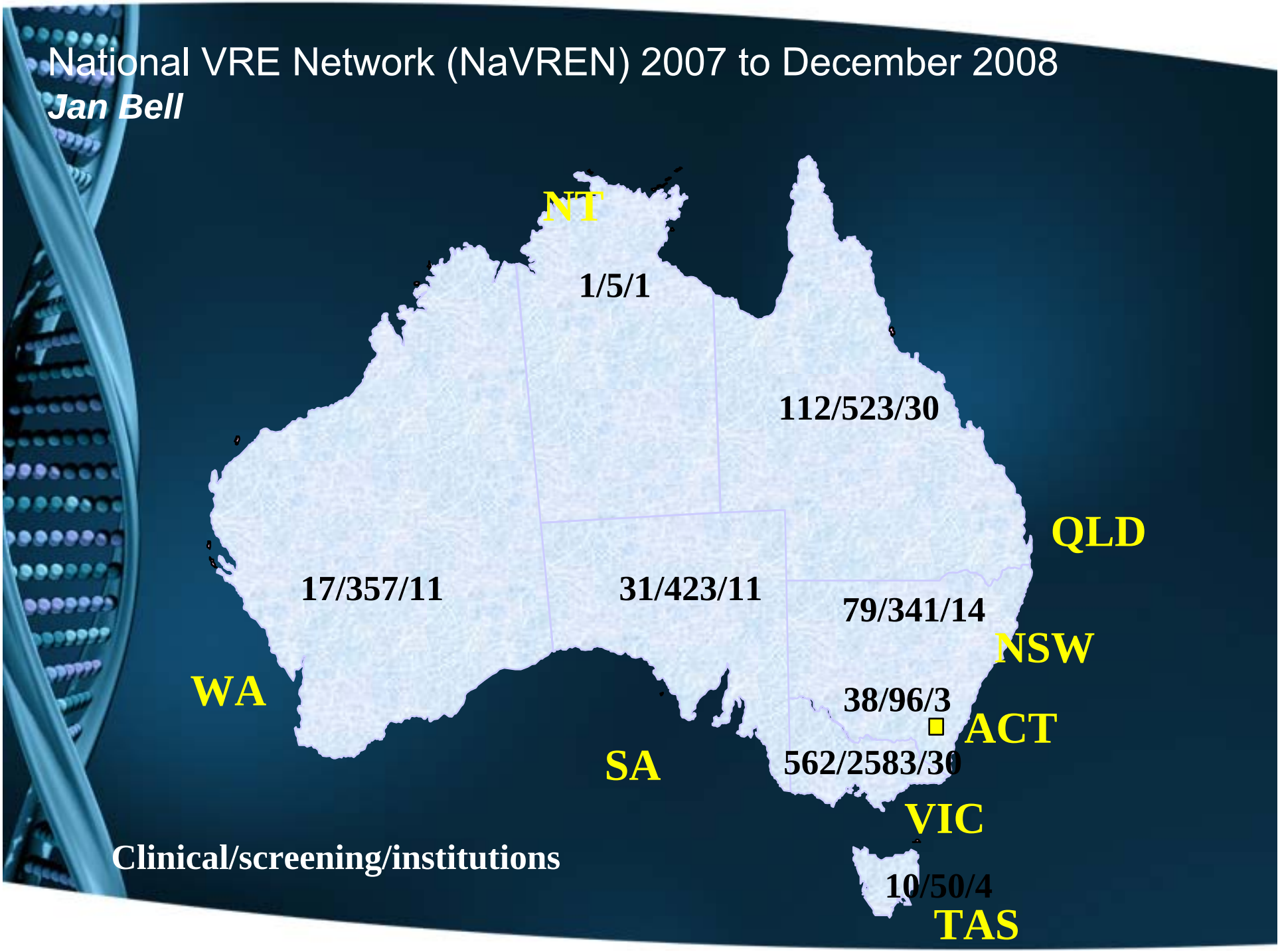
	vanA	*vanB*	*vanA/B*	Negative by vanR PCR	Total
vanA Ph	**110**	3	26	5	144
vanB Ph	0	3	0	0	3
Negative	11	**33**	2	309	355
Total	121	39	28	314	502

Stamper, P. et al. JCM Oct 2007

- In summary the vanR assay is a good screening assay for VRE in a population of mainly *vanA* colonised patients. However patient samples with *vanB* should be confirmed by another method.

National VRE Network (NaVREN) 2007 to December 2008

Jan Bell



NaVREN

Genotype distribution – ‘clinical’

Species	*vanA*	*vanB*	Total	
E. faecium	40	665	705	(82.9%)
E. faecalis	3	135	138	(16.2%)
E. raffinosus	1		1	
Enterococcus spp.	1	5	6	
Total	45	805	850	
	(5.3%)	(94.7%)		

NaVREN

‘Clinical’ Specimen Types

Species	GType	Urine	Wound	Blood	Other	Sterile cavity	Total
E. faecium	*vanA*	27	5	6	2		40
	vanB	309	73	150	82	51	665
E. faecalis	*vanA*	1	1	1			3
	vanB	84	23	8	15	5	135
E. raffinosus	*vanA*				1		1
Total		421	103	165	104	56	805

52.3% 20.5%

Acknowledgments

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Royal Perth Hospital

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- Julie Pearson
- Peta Perry

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- Jan Bell