NVMM Guideline HRMO VRE

Sinnige J.C.[1], Willems R.J.L.[1], Ruijs G.J.H.M.[2], Mascini E.[3], Arends J.P.[4], Troelstra A.[1]
1. Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands
2. Medical Microbiology, Isala, Zwolle, The Netherlands
3. Medical Microbiology, Rijnstate ziekenhuis, Arnhem, The Netherlands
4. Medical Microbiology, University Medical Center Groningen, Groningen, The Netherlands

Deze richtlijn is mede met SKMS-gelden tot stand gekomen

4.2.1 Introduction

Members of the genus *Enterococcus* were classified as Group D *Streptococcus* until 1984, when genomic DNA analysis indicated that a separate genus classification would be appropriate. Shortly after this change in classification the first vancomycin-resistant *Enterococcus faecium* (VRE) isolates were described in the late '80s [1], [2]. Nine different types of vancomycin resistance gene clusters (*van*A, *B*, *C*, *D*, *E*, *G*, *L*, *M*, and *N*) have been characterized on both a phenotypic and a genotypic basis in enterococci [3]. In addition, a tenth vancomycin resistance gene cluster, *vanO*, has recently been described in *Rhodococcus equi* [4]. All these types can correspond to acquired resistance, mainly in *E. faecium* and less frequently in *Enterococcus faecalis* or other *Enterococcus* species, while *van*C is also an intrinsic property of *E. gallinarum* and *E. casseliflavus* [5]. A combination of *vanA* and *vanB* gene clusters together in one *Enterococcus* isolate has also been found. The emergence and dissemination of high-level resistance to vancomycin in enterococci can lead to clinical isolates resistant to most antibiotics. Although enterococci are not highly pathogenic, the incidence of vancomycin resistance among clinical isolates is steadily increasing, and such isolates have become important as nosocomial pathogens and constitute an important reservoir of antibiotic resistance genes. In this guideline, "VRE" is defined as amoxicillin- and vancomycin-resistant *E.feacium*.

4.2.2 Detection of carriage

4.2.2.1 Culture sites

Rectal swabs or stool samples are generally considered the best materials for detection of VRE although the diagnostic accuracy of a rectal swab culture method in identifying gastrointestinal colonization with VRE has a high false-negative rate. Since stool density may vary during an episode of VRE colonization [6], the sensitivity of a rectal swab significantly correlates with higher densities of VRE in stool [7]. Since stool samples are often more difficult to collect, most often rectal swabs are used for VRE surveillance.

4.2.2.2 Number of cultures

One study has shown that on average four rectal swabs, collected on separate days, were needed to detect >90% of carriers [8], other data (to be published soon; Frakking, Sinnige, Tersmette) indicate that five samples collected on separate days detected >95% The VRE carrier status of patients who have already been identified as VRE carrier can be confirmed with fewer cultures as data suggests that almost all positive tested patients remain positive during their hospital stay. In the first stages of colonization, just after a transmission event, VRE detection using swabs can be less sensitive. This is

probably due to the fact that after a transmission event VRE levels in the gut are still low and under the detection limit [9]. Therefore three to five cultures, collected on separate days are recommended to detect VRE carriage in a patient that has been exposed to VRE. Once a patient has been identified as a carrier of VRE, it is not clear how many times culture sets have to be taken to reliably identify loss of carriage. The total number of cultures in one set can be adapted after analysing the local epidemiology. In the Netherlands, patients that are recently transferred from a foreign hospital are screened for HRMO upon admission to the hospital. As long as there is no exposition to VRE reported, this surveillance screening as well as a prevalence screening performed in a non-exposition setting, can pragmatically be performed by means of a single culture.

4.2.2.3 Culture materials and transport

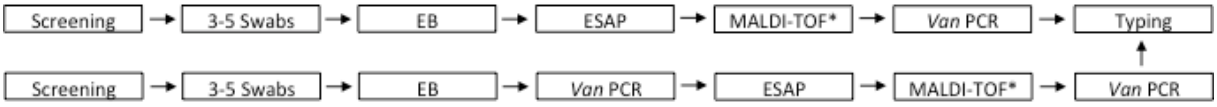
Enterococci can survive for prolonged periods on dry surfaces and under various climatic conditions [10]. Therefore, transport and storage conditions are not critical for detection. Nevertheless, it is recommended to use a transport medium (Amies or Stuart), as this probably increases the detection rate when storage is prolonged. The use of a dry swab is discouraged. It is possible to await processing of several samples from separate days; in that case samples should be kept at 4-8°C until processing.

4.2.2.4 Timing of cultures

There is very little data available on the interval from exposure to VRE to detection in cultures. In one study newly acquired VRE was detected only seven days after exposure from a positive roommate [11]. Therefore, screening contacts of VRE positive patients should last at least until day seven after a last possible transmission event.

4.2.3 Laboratory methods

The recommended strategy for the detection of vancomycin resistance in *E. faecium* is a two-step procedure, and consists of a screening step followed by a genotypic confirmation step. The screening step is based on the reduced susceptibility of VRE to vancomycin. The genotypic confirmation step is based on the detection of the *vanA* or *vanB* gene. An alternative strategy is to first test for *vanA/vanB* genes in the enrichment broth and to confirm the presence of VRE by culture and by performing the genetic confirmation on a colony. *E. faecium* strains that are vancomycin-resistant but negative in the genotypic confirmation (so *vanA/B* gene negative) should be checked for the presence of other vancomycin resistance gene variants.



EB Enrichment broth

ESAP Enterococcus selective agar plate

When screening patients for VRE colonization, 3 to 5 swabs are taken. These swabs are used to inoculate the enrichment broth (EB). From this enrichment broth an enterococcus selective agar plate (ESAP) is inoculated followed by MALDI-TOF-based species identification or conventional biochemical identification(*) of growing colonies, genotypic detection of vancomycin resistance genes and molecular typing of the VRE strain. Alternatively, vancomycin-resistance gene detection is done directly on the enrichment broth, followed by the selective agar plate, MALDI-TOF and typing.

4.2.3.1 Direct molecular detection (vanA, vanB)

The increasing prevalence of VRE has led to increased interest in screening for VRE colonization with PCR. Several commercial genotypic assays are available for VRE screening. The Cepheid GeneXpert *vanA*/*vanB* assay, BD GeneOhm VanR assay, and other commercially available assays have high sensitivity and specificity for detecting *vanA*-positive enterococci, but a low specificity for detecting *vanB*-positive enterococci in faeces samples due to the presence of *vanB* containing commensal anaerobic flora in human faeces [12]–[14]. For in house developed qPCR schemes, the same high sensitivity and specificity was measured as for the commercial tests[15]. Because this high sensitivity and therefore the high negative predictive value, molecular detection of *van*A/*van*B on enrichment broth can be used as a negative screening test [16]. Primer sequences for in house tests are published but should be validated with the equipment used in the local laboratory [17]. Ct values can help to identify the origin of the *vanB* signal. Ct value cutoffs should be set and validated with the primer sets and equipment used in the local laboratory [18].

4.2.3.2 Solid agar media

Conventional media

VRE from clinical specimens with non-selective conventional solid agar media is often overgrown by Gram-negative bacteria and therefore not recommended. A conventional solid agar medium, such as a blood agar plate, can be used as growth control. VRE cultures from non-sterile culture sites should be disapproved when the growth control is negative (i.e. when there is no growth at all, and thus reliability of sampling can be questioned). In addition, a conventional medium may serve as a backup for VRE isolates that are suppressed by the selective agents used in the VRE screening agar (e.g. in *Van*B positive isolates, with low-level vancomycin resistance).

VRE Selective media

Enterococci grow on media containing bile, esculin, and azide and may be differentiated from streptococci by the hydrolysis of esculin in the presence of 40% bile. The combination of esculin and a rather low concentration of bile permits selection by colony morphology because of the visible evidence of a brown-black halo due to esculin hydrolysis. The agar can be supplemented with aztreonam (25 mg/liter) and vancomycin (6 mg/liter) [19]. Chromogenic agars are both selective and differential for detecting vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*. Many of these chromogenic media can be read and finalized within 48 h [20].

4.2.3.3 Broth enrichment

Bile esculin azide broth supplemented with 6 mg of vancomycin per liter [21] can be used for the rapid and selective isolation of VRE from surveillance specimens. Amoxicillin (16 mg per liter) can be added since VRE outbreaks are typically caused by amoxicillin-resistant enterococci (ARE) that acquired resistance for vancomycin [18]. Moreover, amoxicillin limits the growth of amoxicillin-sensitive anaerobic bacteria like *Clostridium* species, which are the most relevant species that can also contain *vanB* genes [12], [14]. In order to perform optimal tailor-made VRE-screening during a specific setting, local adaptations to procedures can be made. In example, in case of a circulating *vanB* VRE strains

expressing low-level vancomycin resistance it is recommended not to add vancomycin to the broth, as this would hamper the growth [22].

4.2.3.4 Pooling of samples

For patients it is recommended to use a separate swab for each culture, and not to pool samples. There is little experience testing pooled samples from the same patient with qPCR detection.

4.2.3.5 Identification

Current routine identification methods for *E. faecium* should be used, as there are no indications that the identification of *E. faecium* is different for vancomycin-susceptible or -resistant strains. Various methods can be used, including biochemical, matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF) or molecular methods.

4.2.3.6 Susceptibility testing

Screening

Routine susceptibility test methods

- Several routine susceptibility test methods can be used for *E. faecium*, including broth dilution, agar dilution, or an automated system, such as VITEK 2 (bioMérieux) or Phoenix (Becton-Dickinson), and E-test. The recommended MIC screening breakpoint for vancomycin is > 4 mg/L [EUCAST 2015] although low-level vancomycin resistance (MIC < 4) is possible in *vanB* isolates. Antimicrobial susceptibility testing methods to detect these low-level vancomycin resistance in enterococci (*vanB* isolates) include disk diffusion and Phoenix 100 since for low-level vancomycin resistance the Phoenix 100 performs significantly better than the Vitek 2 system [23], [24].

Disk diffusion test

- For the disk diffusion test it is recommended to use vancomycin on a Mueller-Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland for 24 hours at 34-36°C, according to the manufacturer's instructions. The recommended zone diameter screening breakpoint for vancomycin is < 12 mm [EUCAST 2015]. Cultures with sharp zone edges and zone diameters of ≥12 mm vancomycin should be reported as susceptible. Cultures with fuzzy zone edges or colonies within the zone should be reported as resistant to vancomycin, even if the zone diameter is ≥12 mm [23].

Confirmation – phenotypic

The MIC for vancomycin can be determined by E-test with 0,5 McFarland on a Muller Hinton agar after 24 hours incubation.

Confirmation – genotypic

Genotypic confirmation should be performed by PCR. Strains which are phenotypically VRE but lack both *van*A and *van*B should be sent to a reference center for the presence of other *van*-genes

Quality control

The following two strains can be used for quality control:

- *Enterococcus faecium* E72 – *vanA*-positive (UMC Utrecht)
- *Enterococcus faecium* E513 – *vanB*-positive (UMC Utrecht)

4.2.4 Contact tracing

4.2.4.1 Adjusting diagnostic methods in case of a 'known' strain

The methods used for targeted screening may be adjusted in order to improve the efficiency of detection of VRE strains that are circulating at the time contact tracing is implemented. This is of special importance in case of a *vanB* VRE with low MICs (<4 mg/L) for vancomycin [22].

4.2.4.2 Molecular typing (AFLP, PFGE, MLST, MLVA)

It is recommended to compare VRE isolates that are detected in contact patients to the isolate of the index patient by (geno)typing. Typing results can be helpful to recognize nosocomial transmission and to control outbreaks. Typing methods that can be used to identify transmission of VRE include amplified fragment length polymorphism (AFLP), pulsed field gel electrophoresis (PFGE) and multi locus sequence typing (MLST). Multiple-locus variable number tandem repeat analysis (MLVA) is considered less discriminatory than MLST and PFGE [25]. Other methods that should be considered are whole genome sequencing (WGS) and WGS coupled with an extended MLST since they provide a higher resolution. A definitive core genome MLST scheme (or MLST+) is currently being developed.

4.2.5 Reporting

4.2.5.1 Laboratory information system

Results from test and controls shall be stored unambiguous in an electronic laboratory information system.

4.2.5.2 Patient information system

Electronic communication of laboratory tests and their results between different information systems (Lab2Lab or Lab2Healthcare) shall meet the requirements as defined in the project "Eenheid van Taal", the defined dataset and HL7 message structure.

4.2.6 Recommendations

Detection of carriage

- VRE screening cultures should include a rectal swab.
- A set of 3-5 specimens from separate days should be used for the targeted screening for VRE carriage, provided that broth enrichment is used. The total number of cultures can be adapted after analysing the local epidemiology.
- Screening contacts of VRE positive patients should last at least until day seven after a last possible transmission event.

- Swabs should be collected in an adequate and locally validated transport medium. The use of dry swabs is not recommended.
- It is recommended to process specimens as soon as possible, although it is possible to await the collection of 3-5 samples from separate days. The samples should be kept at 4-8°C until they are send to the laboratory for further processing.

Laboratory methods

- For targeted VRE screening it is recommended to use a selective broth enrichment in combination with a selective VRE screening agar.
- For chromogenic media the recommended incubation time is 48 hours.
- It is optional to use a conventional solid agar medium as a growth control.
- When a growth control is used VRE cultures from non-sterile culture sites should be disapproved when the growth control is negative.
- For patients it is recommended to use a separate swab for each culture day.
- Current routine identification methods for *E. faecium* should be used, as there are no indications that the identification of *E. faecium* is different for vancomycin-susceptible or -resistant strains.
- The recommended strategy for the detection of vancomycin resistance in *E. faecium* is a two-step procedure, and consists of a screening step followed by a genotypic confirmation step.
- Routine susceptibility test methods to screen for vancomycin resistance are broth dilution, agar dilution, or an automated system. Antimicrobial susceptibility testing methods to detect low-level vancomycin resistance in enterococci (*vanB* isolates) include disk diffusion and Phoenix 100 since for low-level vancomycin resistance the Phoenix 100 performs significantly better than the Vitek 2 system
- The recommended MIC screening breakpoint for vancomycin is > 4 mg/L.
- Cultures with fuzzy zone edges or colonies within the zone should be reported as resistant to vancomycin, even if the zone diameter is ≥12 mm
- PCR based methods should be used for the detection of the *vanA*/*vanB* gene.
- *E. faecium* strains that are vancomycin-resistant but negative in the *vanA*/*vanB* genetic confirmation test, should be tested for the presence of other vancomycin-resistance genes.

Contact tracing

- For contact tracing it is recommended to confirm that the methods used for targeted screening are able to detect strains that are ‘known’ to circulate at the time contact tracing is implemented.
- It is recommended to compare VRE isolates that are detected in contact patients to the isolate of the index patient by (geno) typing of strains.

Reporting

- Results from test and controls shall be stored unambiguous in an electronic laboratory information system.

- Electronic communication of laboratory tests and their results between different information systems (Lab2Lab or Lab2Healthcare) shall meet the requirements as defined in the project "Eenheid van Taal", the defined dataset and HL7 message structure.
- The antibiogram to be reported in the patient information system should be in accordance with the EUCAST clinical breakpoints [EUCAST 2015]
- VRE test results should be reported to the treating physician and infection control department as soon as results are available.

4.2.7 Suggestions for future research

- How many negative cultures should be taken to declare someone negative?
- What is the optimal timing of contact screening after a positive identified patient?
- Duration of carriership; how many sets have to be tested before a patient can be unlabeled?
- Is it possible to pool samples?

[1] R. Leclercq, E. Derlot, J. Duval, and P. Courvalin, "Plasmid-mediated resistance to vancomycin and teicoplanin in Enterococcus faecium," *N. Engl. J. Med.*, vol. 319, no. 3, pp. 157–161, Jul. 1988.

[2] A. H. Uttley, C. H. Collins, J. Naidoo, and R. C. George, "Vancomycin-resistant enterococci," *Lancet*, vol. 1, no. 8575–6, pp. 57–58, Jan. 1988.

[3] V. Cattoir and R. Leclercq, "Twenty-five years of shared life with vancomycin-resistant enterococci: is it time to divorce?," *J. Antimicrob. Chemother.*, vol. 68, no. 4, pp. 731–742, Apr. 2013.

[4] D. D. Gudeta, A. Moodley, V. Bortolaia, and L. Guardabassi, "vanO, a new glycopeptide resistance operon in environmental Rhodococcus equi isolates," *Antimicrob. Agents Chemother.*, vol. 58, no. 3, pp. 1768–1770, 2014.

[5] P. Courvalin, "Vancomycin Resistance in Gram-Positive Cocci," *Clin. Infect. Dis.*, vol. 42, no. Supplement 1, pp. S25–S34, Jan. 2006.

[6] C. J. Donskey, C. K. Hoyen, S. M. Das, M. S. Helfand, and M. T. Hecker, "Recurrence of vancomycin-resistant Enterococcus stool colonization during antibiotic therapy," *Infect. Control Hosp. Epidemiol. Off. J. Soc. Hosp. Epidemiol. Am.*, vol. 23, no. 8, pp. 436–440, Aug. 2002.

[7] E. M. C. D'Agata, S. Gautam, W. K. Green, and Y. Tang, "High Rate of False-Negative Results of the Rectal Swab Culture Method in Detection of Gastrointestinal Colonization with Vancomycin-Resistant Enterococci," *Clin. Infect. Dis.*, vol. 34, no. 2, pp. 167–172, Jan. 2002.

[8] J. W. Pearman, "2004 Lowbury Lecture: the Western Australian experience with vancomycin-resistant enterococci - from disaster to ongoing control," *J.Hosp.Infect.*, vol. 63, no. 0195–6701 (Print), pp. 14–26, mei 2006.

[9] R. Kaki MD, Y. Yu MD, C. O'Neill MLT, C. Lee MD, D., MD, MSc Mertz, and H. H. S. I. P. and C. Team, "Vancomycin-Resistant Enterococcus (VRE) Transmission and Risk Factors in Contacts of VRE Carriers," *Infect. Control Hosp. Epidemiol.*, vol. 35, no. 7, pp. 876–879, Jul. 2014.

[10] J. H. T. Wagenvoort, E. I. G. B. De Brauwer, R. J. R. Penders, R. J. Willems, J. Top, and M. J. Bonten, "Environmental survival of vancomycin-resistant Enterococcus faecium," *J. Hosp. Infect.*, vol. 77, no. 3, pp. 282–283, Mar. 2011.

[11] M., MHSc Qian Zhou, A. Christine Moore, R. Sarah Eden, Ms. Agnes Tong, M., FRCPC Allison McGeer, and M. S. H. I. C. Team, "Factors Associated with Acquisition of Vancomycin-Resistant Enterococci (VRE) in Roommate Contacts of Patients Colonized or Infected with VRE in a Tertiary Care Hospital •," *Infect. Control Hosp. Epidemiol.*, vol. 29, no. 5, pp. 398–403, May 2008.

[12] S. A. Ballard, K. K. Pertile, M. Lim, P. D. R. Johnson, and M. L. Grayson, "Molecular characterization of vanB elements in naturally occurring gut anaerobes," *Antimicrob. Agents Chemother.*, vol. 49, no. 5, pp. 1688–1694, May 2005.

[13] P. D. Stamper, M. Cai, C. Lema, K. Eskey, and K. C. Carroll, "Comparison of the BD GeneOhm VanR assay to culture for identification of vancomycin-resistant enterococci in rectal and stool specimens," *J. Clin. Microbiol.*, vol. 45, no. 10, pp. 3360–3365, Oct. 2007.

[14] S. A. Ballard, E. A. Grabsch, P. D. R. Johnson, and M. L. Grayson, “Comparison of Three PCR Primer Sets for Identification of vanB Gene Carriage in Feces and Correlation with Carriage of Vancomycin-Resistant Enterococci: Interference by vanB-Containing Anaerobic Bacilli,” *Antimicrob. Agents Chemother.*, vol. 49, no. 1, pp. 77–81, Jan. 2005.
[15] M. S. Mehta, S. M. Paule, D. M. Hacek, R. B. Thomson, K. L. Kaul, and L. R. Peterson, “Optimization of a Laboratory-Developed Test Utilizing Roche Analyte-Specific Reagents for Detection of Staphylococcus aureus, Methicillin-Resistant S. aureus, and Vancomycin-Resistant Enterococcus Species,” *J. Clin. Microbiol.*, vol. 46, no. 7, pp. 2377–2380, Jul. 2008.
[16] M. Bongaerts, E. J. van Hannen, K. C. J. Frijns, M. E. L. M. Manders, and E. R. Heddema, “Screening with VanA/B PCR to detect vancomycin resistant Enterococci using the Siemens VERSANT kPCR system,” presented at the ECCMID, Barcelona, 2014.
[17] S. Dutka-Malen, S. Evers, and P. Courvalin, “Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR,” *J.Clin.Microbiol.*, vol. 33, no. 0095–1137 (Print), pp. 24–27, Jan. 1995.
[18] X. Zhou, J. P. Arends, G. A. Kampinga, H. M. Ahmad, B. Dijkhuizen, P. van Barneveld, J. W. A. Rossen, and A. W. Friedrich, “Evaluation of the Xpert vanA/vanB assay using enriched inoculated broths for the direct detection of vanB VRE,” *J. Clin. Microbiol.*, pp. JCM.01125–14, Oct. 2014.
[19] E. M. Mascini, K. P. Jalink, T. E. M. Kamp-Hopmans, H. E. M. Blok, J. Verhoef, M. J. M. Bonten, and A. Troelstra, “Acquisition and Duration of Vancomycin-Resistant Enterococcal Carriage in Relation to Strain Type,” *J. Clin. Microbiol.*, vol. 41, no. 12, pp. 5377–5383, Dec. 2003.
[20] N. Suwantarat, A. Roberts, J. Prestridge, R. Seeley, S. Speser, C. Harmon, C. Zhang, S. Henciak, P. D. Stamper, T. Ross, and K. C. Carroll, “Comparison of Five Chromogenic Media for Recovery of Vancomycin-Resistant Enterococci from Fecal Samples,” *J. Clin. Microbiol.*, vol. 52, no. 11, pp. 4039–4042, Nov. 2014.
[21] K. G. van Horn, C. A. Gedris, and K. M. Rodney, “Selective isolation of vancomycin-resistant enterococci,” *J. Clin. Microbiol.*, vol. 34, no. 4, pp. 924–927, Apr. 1996.
[22] G. Werner, I. Klare, C. Fleige, U. Geringer, W. Witte, H.-M. Just, and R. Ziegler, “Vancomycin-resistant vanB-type Enterococcus faecium isolates expressing varying levels of vancomycin resistance and being highly prevalent among neonatal patients in a single ICU,” *Antimicrob. Resist. Infect. Control*, vol. 1, no. 1, p. 21, 2012.
[23] K. Hegstad, C. G. Giske, B. Haldorsen, E. Matuschek, K. Schønning, T. M. Leegaard, G. Kahlmeter, A. Sundsfjord, and NordicAST VRE Detection Study Group, “Performance of the EUCAST disk diffusion method, the CLSI agar screen method, and the Vitek 2 automated antimicrobial susceptibility testing system for detection of clinical isolates of Enterococci with low- and medium-level VanB-type vancomycin resistance: a multicenter study,” *J. Clin. Microbiol.*, vol. 52, no. 5, pp. 1582–1589, May 2014.
[24] S. Pendle, P. Jelfs, T. Olma, Y. Su, N. Gilroy, and G. L. Gilbert, “Difficulties in detection and identification of Enterococcus faecium with low-level inducible resistance to vancomycin, during a hospital outbreak,” *Clin. Microbiol. Infect.*, vol. 14, no. 9, pp. 853–857, Sep. 2008.
[25] G. Werner, I. Klare, and W. Witte, “The current MLVA typing scheme for Enterococcus faecium is less discriminatory than MLST and PFGE for epidemic-virulent, hospital-adapted clonal types,” *BMC Microbiol.*, vol. 7, p. 28, 2007.