Quality of molecular detection of vancomycin resistance in enterococci : results of 6 consecutive years of Quality Control for Molecular Diagnostics (QCMD) external quality assessment

Quality of molecular detection of vancomycin resistance in Enterococci: results of 6 consecutive years of Quality Control for Molecular Diagnostics (QCMD) external quality assessment

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Abstract

Purpose: The quality of PCR to detect vancomycin-resistant enterococci (VRE) was evaluated by analysing their performance in 6 consecutive external quality assessment (EQA) schemes, organized annually since 2013 by Quality Control for Molecular Diagnostics.

Methods: VRE EQA panels consisted of 12-14 heat-inactivated samples. Sensitivity was tested with *vanA* positive *Enterococcus faecium* (*E. faecium)*, *vanB* positive *E. faecium, E. faecalis* or *E. gallinarum* or *vanC* positive *E. gallinarum* in different concentrations. Vancomycin-susceptible Enterococci, *Staphylococcus aureus* or sample matrix were used to study the specificity. Participants were asked to report the VRE resistance status of each sample.

Results: The detection rate of *vanA* positive samples was already 95% in the 2013 EQA panel (range: 94-97%) and remained stable over the years. The 2013 detection rate of *vanB* positive samples was 82% but increased significantly by more than 10% in subsequent years (96% in 2014, 95% in 2015, 92% in 2016 and 93% in 2017/2018, $p<0.05$). The *vanC* detection rate by the limited number of assays specifically targeting this gene was lower compared to *vanA/B* (range: 55-89%). The number of false positives in the true-negative sample (8% in 2013 to 1.4% in 2018) as well as the van-gene-negative bacterial samples (4% in 2013 to 0% in 2018) declined over the years.

Conclusions: In the 6 years of VRE proficiency testing to date, the detection of *vanA* positive strains was excellent and an increased sensitivity in *vanB* detection as well as an increase in specificity was observed. Commercial and in-house assays performed equally well.

Keywords

VRE, proficiency testing, vancomycin resistance, molecular diagnostics

Introduction

Vancomycin-resistant enterococci (VRE) are a significant cause of healthcare-acquired infections due to their colonization potential and environmental persistence [1]. Adequate infection control of VRE strongly depends on the speed and quality of (molecular) identification strategies used by clinical microbiology laboratories. Culture methods are primarily used for the detection of VRE but they have the disadvantage of prolonged incubation periods, which has been improved partly by the use of selective chromogenic media [2]. Nucleic acid amplification techniques (NAATs) have the potential to further reduce the time to identification as well as improve the sensitivity.

Over the last decade, a range of commercial and in-house developed NAATs have been introduced, targeting the glycopeptide resistance genes (*van* genes). Currently, 1 intrinsic (*vanC)* and 8 acquired (*vanA*, *vanB*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM* and *vanN*) *van* genes have been described [3]. *VanA* is responsible for the majority of human cases of VRE globally, mainly carried by *Enterococcus faecium* (*E. faecium*) [1], while *vanB* carrying isolates are less prevalent but are also found throughout the world [4]. The presence of *vanC* genes, encoding for low levels of vancomycin resistance, are an intrinsic property of *Enterococcus gallinarum* (*E. gallinarum*) and *Enterococcus casseliflavus* (*E. casseliflavus*) and detection of *vanC* genes can therefore be used for confirmation of their identification [5]. From an epidemiological and infection control perspective these species are not significant. Therefore, the detection of *vanC* genes is not included in the majority of commercially available or in-house assays, with most NAATs targeting only *vanA* or *vanA* and *vanB*.

The introduction of NAATs for VRE identification necessitated the requirement of appropriate quality control as differences in the performance of commercial VRE assays had been

64 described [6]. Furthermore, participation in external quality assessment (EQA) programs is an
65 essential requirement for accreditation of medical laboratories (ISO15189 or equivalent) as it
66 allows comparison of the performance of diagnostic tests with other laboratories or methods
67 [7]. The VRE pilot EQA was introduced in 2013, sent out yearly and coordinated by Quality
68 Control for Molecular Diagnostics (QCMD) in Glasgow, Scotland. In this study, we compared
69 the participant characteristics, the applied molecular assays and their performance in 6
70 consecutive VRE EQA panels.

71

72 **Materials and methods**

73 VRE EQA panels consisted of 12 to 14 samples: 3 or 4 *vanA* positive (*E. faecium* strains
74 LMG16165 or IOWA1), 4 *vanB* positive (*E. faecium* strain IOWA2, *Enterococcus faecalis* (*E.*
75 *faecalis*) strain ATCC51299), 1 combined *vanB* and *vanC* positive (*E. gallinarum* characterized
76 by the Belgian VRE reference laboratory, Antwerp University Hospital), 1 *vanC* positive (*E.*
77 *gallinarum* strain LMG16289) and 3 or 4 negative samples (sample matrix or glycopeptide
78 susceptible *Enterococcus* species or *Staphylococcus aureus*). Samples were prepared in brain
79 heart infusion matrix and all bacterial samples were heat-inactivated for 10 min at 100°C. The
80 concentration of VRE in the different samples varied from 10^3 to 10^7 CFU/ml. Panels were
81 distributed on dry ice to 44-71 participating laboratories in 14-21 countries (Table 1) along
82 with detailed sample processing instructions. Participants were given 4 weeks to analyse the
83 samples and to report their results to QCMD via their online data collection system.
84 Participants were asked in the first instance to report the VRE resistance status of each sample
85 by indicating whether it was positive or negative for vancomycin antibiotic resistance. If
86 resistance was detected, laboratories were asked to specify the resistance gene identified (i.e.

vanA, vanB, vanC). QCMD analysed the data and results were anonymously released to all participants in a detailed EQA final report. The individual sample codes, as presented in Tables 3-6, start with 'VRE', followed by the year of distribution and a random serial number.

Differences in detection rates of *vanA*, *vanB* or *vanC* positive samples and differences in the use of in-house versus commercial tests between the different EQA panels over the years were analysed with Kruskal–Wallis and Mann–Whitney U tests. Wilcoxon Signed Ranks Test was used to investigate differences in detection rates by commercial versus in-houses NAATs. Statistical analyses were performed with SPSS statistics 21.0 software.

Results

General observations

Over the 6 years of VRE EQA the percentage of datasets generated using commercial assays (26% in 2013 versus 50% in 2018) increased significantly ($p<0.05$ between 2013 and 2017/2018) compared to the percentage of datasets generated using in-house assays (74% in 2013 versus 50% in 2018), which is a pattern seen across the molecular diagnostics field as increasing regulatory requirements come into force [8]. Nonetheless, the in-house assays remained the most widely used in these VRE programs until 2017 (Table 1). Up to 19 different commercial PCR methods were reported with increased diversity over the years, reflecting the expanding spectrum of commercially available assays. The most frequently used commercial PCR methods are the Xpert vanA and Xpert vanA/vanB assays (Cepheid), representing 65% of all commercial assays in the 2018 EQA panel (Table 2). For both the in-

house and commercial assays, real-time PCR was applied more often than conventional PCR, 89% and 97% in 2018, respectively (Table 1).

Detection of *vanA*

The positivity rates for all the samples containing *vanA* positive strains over the years are presented in Fig. 1a and Table 3. In 2013, the detection rate was already high at 95% (range: 92-97%). This mean percentage did not change significantly over the following years (95% in 2014, 97% in 2015, 95% in 2016 and 2017 and 94% in 2018). It should be noted that the 2013 EQA panel contained the highest concentration of bacterial cells (10^5-10^7CFU/ml), compared to the subsequent years (10^3-10^5CFU/ml). The lowest *vanA* positivity rate in all panels was 81% for VRE14-10. This is a sample containing the lowest concentration (10^3 CFU/ml). However, the detection rate increased for similar samples in the following EQA panels to 92% in 2015 (VRE15-02), 93% in 2016 (VRE16-10), 92% in 2017 (VRE17-01) and 90% in 2018 (VRE18-07) indicating an increased sensitivity of both in-house and commercial assays in recent years. Nevertheless, a decline in sensitivity for this low-concentrated *vanA* positive sample was observed for the in-house assays in 2018 (83% detection of vancomycin resistance for VRE18-07 compared to 97% for the commercial assays). In most datasets, besides the vancomycin resistance status, *vanA* could be identified as the resistance gene involved.

Detection of *vanB*

The mean positivity rate for the detection of vancomycin resistance in *vanB* positive strains was 82% (range: 72-87%) in 2013. This rate increased significantly to 96% in 2014 (range: 92-97%, p=0.01) and remained above 90% in the subsequent years (95% in 2015, p=0.01 versus 2013, 92% in 2016, p=0.03 versus 2013, 93% in 2017, p=0.03 versus 2013 and 2018, p=0.01

versus 2013) (Fig. 1b and Table 4). Moreover, the 2013 panel contained the highest concentration range (10^5-10^7CFU/ml), compared to the subsequent years (10^4-10^6CFU/ml) indicating a definite increase in the performance of the different assays to detect the *vanB* gene. There was no statistically significant difference in detection rate between the last five EQA panels ($p>0.05$). The combined results of *vanA* and *vanB* detection indicate no statistically significant difference in the detection of both genes over the different EQA panels (mean positivity rates: 88% in 2013, 93% in 2016 and 2018, 94% in 2017 and 95% in 2014). However, the higher bacterial load in the first EQA panel for both *vanA* and *vanB* containing strains should be kept in mind. Similar to the detection of *vanA* positive strains, also for *vanB* positive strains most participants could identify the correct resistance gene.

Detection of *vanC*

The low detection rates for vancomycin resistance in the EQA samples containing only the *vanC* gene (VRE13-06, VRE14-06, VRE15-11, VRE16-04, VRE17-05 and VRE18-02), ranging from 11 to 36% for all datasets, is explained by the absence of this target in the majority of commercially available or in-house NAATs (Table 5). Only the following commercial assays, GenoType Enterococcus 12 and 96 (Hain Lifescience) and Vancomycin Resistance kit (BioGX), target the *vanC* gene. Detection rates of vancomycin resistance for the *vanC* positive samples ranged from 11 to 36 % and did not differ significantly between the different EQA panels ($p=0.95$). In the combined *vanB*/*vanC* positive *E. gallinarum*, the detection rate was logically much higher (range: 87-97%) and comparable with the *vanB* detection rates.. Taking into consideration the results of the assays able to detect *vanC*, detection rates for the *vanC* positive samples ranged from 55% (VRE18-02) to 89% (VRE13-06) and from 90% (VRE15-03) to 100% (all other EQA panels) for the combined *vanB*/*vanC* positive *E. gallinarum.*

Commercial versus in-house NAATs

There was no statistically significant difference in the detection of *vanA* and *vanB* by commercial versus in-house NAATs (p=0.81 for *vanA*, p=0.96 for *vanB* and p=0.09 for *vanC*). Regarding the detection of *vanA* positive strains it is remarkable that in the 2017 EQA panel the in-house assays performed almost perfectly with only 1 of the 35 laboratories missing 1 out of 3 *vanA* positive samples, reaching a *vanA* detection rate of 99% compared to 91% for the commercial assays. The exact opposite trend was observed the year thereafter with an almost flawless detection of vancomycin resistance in *vanA* positive samples by the commercial assays (99%) compared to 89% for the in-house assays. A perfect score was obtained by the commercial assays in the 2014 EQA panel for the detection of *vanB* positive samples. This was also the year in which no Xpert vanA assays (Cepheid) were being used. This FDA-approved assay was shown not to detect *vanB* positive strains in contrast to the CE-labeled Xpert vanA/vanB assay [9]. However, considering the high positivity rates observed for *vanB* positive strains also in years where Xpert vanA assays were more frequently used, one can assume that either the Xpert vanA assay has been modified over the years to also detect *vanB* positive strains or that participants fail to correctly register the assay type on the QCMD online data collection system. The latter seems the most plausible explanation.

Specificity results

Regarding the true negative samples (sample matrix), the false positivity rate declined over the observed period (Table 6). The false positivity rate in samples containing vancomycin susceptible *Enterococci* spp remained low in all panels (maximum 3% and even 0% in both samples of the 2014 and 2018 EQA panel). However, in the 2013 VRE EQA panel, one of the negative samples (VRE13-07) containing a vancomycin susceptible *E. faecium*, was found

positive in 51% of all datasets (62% of the in-house and 20% of the commercial tests). This might be explained by the presence of vancomycin-susceptible *vanA*-positive *E. faecium* [10,11] or non-specific amplification. Nonetheless, the strain was excluded from further EQA panels and the results of this sample should be interpreted with care.

The number of false positives in *Staphylococcus aureus* samples declined over the years starting from 5.1% in 2013 to 0% in 2018 (Table 6). Overall, the levels of incorrect determination were sometimes even lower in negative samples containing *Enterococcus* spp or other bacteria than in the true negative sample (sample matrix) indicating good specificity of the assays.

Discussion

Quality control of molecular diagnostics is crucial in maintaining high-quality clinical care. This is the first report on consecutive proficiency testing results for the molecular detection of VRE. We can conclude that the molecular detection of *vanA* and *vanB* containing Enterococci is reliable. Most of the results are generated by in-house tests but commercially available kits are increasingly being used. All tests performed equally, without any statistically significant difference in sensitivity or specificity between commercial and in-house testing. Since 2013 over 92% of datasets correctly identified vancomycin resistance in *vanA* positive samples, with the most pronounced discrepancy observed in a low-concentration sample (81% positivity rate in VRE14-10). Eighty-two percent of datasets returned in the 2013 VRE EQA identified vancomycin resistance in *vanB* positive samples. This percentage increased significantly by more than 10% in the subsequent years. The low detection rates for vancomycin resistance in the EQA samples containing only the *vanC* gene can be explained by

the absence of this target in the majority of tests. False positivity rates both in the 'true-negative' (sample matrix) and 'specificity' (glycopeptide susceptible *Enterococcus* species or *Staphylococcus aureus*) samples also decreased over the years. Again, this was not statistically significant.

To sustain and further improve the quality of VRE molecular detection, the availability of a VRE EQA program should be maintained and future EQA distributions should contain more challenging samples including other sample matrices because the specificity of VRE detection will be highly influenced by the presence of *vanB*-containing anaerobic bacilli if faecal samples are being tested [12].

Conflict of interest The authors declare that they have no conflict of interest.

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Table legends

Table 1 Overview of program details, reponse rates and assays used for detection of vancomycin resistance over the 6 years of VRE proficiency testing

Table 2 Overview of commercial assays used over the 6 years of VRE proficiency testing

Table 3 Qualitative results for all vanA positive samples in the 6 years of VRE proficiency testing

Table 4 Qualitative results for all vanB positive samples in the 6 years of VRE proficiency testing

Table 5 Qualitative results for all vanC positive samples in the 6 years of VRE proficiency testing

Table 6 False positive results for all vanA/B/C negative samples in the 6 years of VRE proficiency testing

Figure legends

Fig. 1 Detection rates of *vanA* (a) and *vanB* (b) positive samples over the years. (Error bars indicate the 95% confidence intervals, * $p<0.05$)

Table 1: overview of program details, reponse rates and assays used for detection of vancomycin resistance over the 6 years of VRE proficiency testing

	2013	2014	2015	2016	2017	2018
N° participants	44	44	55	65	68	71
N° countries	16	14	17	21	21	21
N° participants returning results	34 (77%)	35 (80%)	46 (84%)	62 (95%)	60 (88%)	58 (82%)
N° officially withdrawing participants	6 (14%)	3 (7%)	6 (11%)	1 (2%)	2 (3%)	8 (11%)
N° participants not returning results	4 (9%)	6 (14%)	3 (5%)	2 (3%)	6 (9%)	5 (7%)
N° returned datasets	39	37	49	67	66	70
N° commercial assays	10 (26%)	11 (30%)	18 (37%)	30 (45%)	31 (47%)	35 (50%)
○ **Conventional PCR**	2	2	3	3	1	1
○ **Real-time PCR**	8	9	15	27	30	34
N° in-house assays	29 (74%)	26 (70%)	31 (63%)	37 (55%)	35 (53%)	35 (50%)
○ **Conventional PCR**	7	5	7	9	3	4
○ **Real-time PCR**	22	21	24	28	32	31

279 Table 2: Overview of commercial assays used over the 6 years of VRE proficiency testing

	2013	2014	2015	2016	2017	2018
Total number	10	11	18	30	31	35
Conventional PCR	2 (20%)	2 (18%)	3 (17%)	3 (10%)	1 (3%)	1 (3%)
○ **GenoType Enterococcus 12 (Hain Lifescience)**	2	1	2	1	0	0
○ **GenoType Enterococcus 96 (Hain Lifescience)**	0	1	1	2	1	1
Real-time PCR	8 (80%)	9 (82%)	15 (83%)	27 (90%)	30 (97%)	34 (97%)
○ **Xpert vanA (Cepheid)**	1	0	5	10	7	6
○ **Xpert vanA/vanB (Cepheid)**	5	6	6	9	11	16
○ **LightCycler VRE Detection kit (Roche)**	1	1	-	-	1	1
○ **Sentosa SA vanA/vanB PCR Test (Vela Diagnostics)**	1	1	-	-	-	-
○ **Artus vanR QS-RGQ Kit (Qiagen)**	-	1	1	1	-	-
○ **GeneProof VRE PCR kit (GeneProof)**	-	-	1	1	1	1
○ **Matriks IDT assay (Integrated DNA Technologies)**	-	-	1	1	1	1
○ **Sepsis Flow CHIP (Master Diagnostica)**	-	-	1	1	2	-
○ **Bosphore VRE detection kit (Anatolia Geneworks)**	-	-	-	1	-	-
○ **FilmArray Blood Culture Identification Panel (BioMérieux)**	-	-	-	1	2	-
○ **Magicplex Sepsis Real-time test (Seegene)**	-	-	-	1	-	1
○ **Ion Xpress Plus Fragment Library kit (Thermo Fisher)**	-	-	-	1	1	1
○ **Vancomycin Resistance kit (BioGX)**	-	-	-	-	1	2
○ **Fluorion VRE QLP (Iontek)**	-	-	-	-	1	-
○ **Viasure Vancomycin Resistance Detection Kit (CerTest)**	-	-	-	-	1	2
○ **VRE Real Time PCR (Vitassay)**	-	-	-	-	1	1
○ **Amplidiag CarbaR+VRE (Mobidiag)**	-	-	-	-	-	2

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Table 3: Qualitative results for all vanA positive samples in the 6 years of VRE proficiency testing

Code	Content	Concentration (CFU/ml)	All datasets			Commercial assays		In-house assays	
			n	%	detected genes	n	%	n	%
VRE13-01	*E. faecium* (LMG16165)	1.0 x 10^5	36/39	92	34 vanA, 1 vanC, 1 NS	9/10	90	27/29	93
VRE13-02	*E. faecium* (IOWA1)	1.0 x 10^5	38/39	97	37 vanA, 1 NS	10/10	100	28/29	97
VRE13-10	*E. faecium* (LMG16165)	1.0 x 10^6	36/39	92	34 vanA, 1 vanC, 1 NS	9/10	90	27/29	93
VRE13-13	*E. faecium* (LMG16165)	1.0 x 10^7	38/39	97	37 vanA, 1 NS	10/10	100	28/29	97
VRE14-02	*E. faecium* (LMG16165)	1.0 x 10^5	37/37	100	37 vanA	11/11	100	26/26	100
VRE14-07	*E. faecium* (IOWA1)	1.0 x 10^4	37/37	100	37 vanA	11/11	100	26/26	100
VRE14-10	*E. faecium* (LMG16165)	1.0 x 10^3	30/37	81	30 vanA	8/11	73	22/26	85
VRE14-12	*E. faecium* (LMG16165)	1.0 x 10^4	36/37	97	36 vanA	10/11	91	26/26	100
VRE15-02	*E. faecium* (LMG16165)	1.0 x 10^3	45/49	92	44 vanA, 1 vanB	17/18	94	28/31	90
VRE15-08	*E. faecium* (IOWA1)	1.0 x 10^4	48/49	98	48 vanA	18/18	100	30/31	97
VRE15-12	*E. faecium* (LMG16165)	1.0 x 10^4	49/49	100	49 vanA	18/18	100	31/31	100
VRE16-02	*E. faecium* (LMG16165)	1.0 x 10^4	65/67	97	65 vanA	29/30	97	36/37	97
VRE16-09	*E. faecium* (IOWA1)	1.0 x 10^4	64/67	96	63 vanA, 1 vanA/B	29/30	97	35/37	95
VRE16-10	*E. faecium* (LMG16165)	1.0 x 10^3	62/67	93	62 vanA	27/30	90	35/37	95
VRE17-01	*E. faecium* (LMG16165)	1.0 x 10^3	61/66	92	60 vanA, 1 vanA+B	27/31	87	34/35	97
VRE17-06	*E. faecium* (LMG16165)	1.0 x 10^4	63/66	95	62 vanA, 1 vanA+B	28/31	90	35/35	100
VRE17-11	*E. faecium* (IOWA1)	1.0 x 10^4	65/66	98	63 vanA, 1 vanA+B, 1 vanA/B	30/31	97	35/35	100
VRE18-07	*E. faecium* (LMG16165)	1.0 x 10^3	63/70	90	63 vanA	34/35	97	29/35	83
VRE18-08	*E. faecium* (IOWA1)	1.0 x 10^4	67/70	96	67 vanA	35/35	100	32/35	91
VRE18-12	*E. faecium* (LMG16165)	1.0 x 10^4	67/70	96	67 vanA	35/35	100	32/35	91
	TOTAL		**1007/1060**	**95.0**		**405/426**	**94.6**	**602/634**	**95.0**

NS: not specified

283 Table 4: Qualitative results for all *vanB* positive samples in the 6 years of VRE proficiency testing

Code	Content	Concentration (CFU/ml)	All datasets			Commercial assays		In-house assays	
			n	%	detected genes	n	%	n	%
VRE13-05	*E. faecalis* (ATCC51299)	1.0×10^7	32/39	82	30 vanB, 1 vanA+B, 1 NS	8/10	80	24/29	83
VRE13-08	*E. faecalis* (ATCC51299)	1.0×10^6	34/39	87	31 vanB, 1 vanB+C, 1 NS	8/10	80	26/29	90
VRE13-09	*E. gallinarum* (ENT20120142)*	1.0×10^6	34/39	87	25 vanB, 8 vanB+C, 1 NS	8/10	80	26/29	90
VRE13-11	*E. faecium* (IOWA2)	1.0×10^5	32/39	82	31 vanB, 1 NS	8/10	80	24/29	83
VRE13-14	*E. faecalis* (ATCC51299)	1.0×10^5	28/39	72	27 vanB, 1 NS	7/10	70	21/29	72
VRE14-01	*E. faecalis* (equivalent to ATCC51299)	1.0×10^5	36/37	97	36 vanB	11/11	100	25/26	96
VRE14-04	*E. gallinarum* (ENT20120142)*	1.0×10^5	36/37	97	28 vanB, 8 vanB+C	11/11	100	25/26	96
VRE14-09	*E. faecium* (IOWA2)	1.0×10^4	35/37	95	34 vanB, 1 vanA	11/11	100	24/26	92
VRE14-11	*E. faecalis* (equivalent to ATCC51299)	1.0×10^4	34/37	92	34 vanB	11/11	100	23/26	88
VRE14-13	*E. faecalis* (equivalent to ATCC51299)	1.0×10^6	36/37	97	35 vanB, 1 vanA+B	11/11	100	25/26	96
VRE15-01	*E. faecium* (IOWA2)	1.0×10^4	46/49	94	45 vanB, 1 vanA	17/18	94	29/31	94
VRE15-03	*E. gallinarum* (ENT20120142)*	1.0×10^5	47/49	96	38 vanB, 8 vanB+C, 1 vanC	18/18	100	29/31	94
VRE15-04	*E. faecalis* (equivalent to ATCC51299)	1.0×10^5	47/49	96	47 vanB	18/18	100	29/31	94
VRE15-07	*E. faecalis* (equivalent to ATCC51299)	1.0×10^4	45/49	92	45 vanB	17/18	94	28/31	90
VRE15-10	*E. faecalis* (equivalent to ATCC51299)	1.0×10^6	47/49	96	46 vanB, 1 vanA+B	17/18	94	30/31	97
VRE16-01	*E. faecium* (IOWA2)	1.0×10^4	62/67	93	62 vanB	26/30	87	36/37	97
VRE16-03	*E. faecalis* (equivalent to ATCC51299)	1.0×10^6	64/67	96	61 vanB, 1 vanA, 1 vanA+B, 1 vanA/B	28/30	93	36/37	97
VRE16-07	*E. faecalis* (equivalent to ATCC51299)	1.0×10^4	58/67	87	58 vanB	25/30	83	33/37	89
VRE16-11	*E. faecalis* (equivalent to ATCC51299)	1.0×10^5	62/67	93	61 vanB, 1 vanA/B	26/30	87	36/37	97
VRE16-12	*E. gallinarum* (ENT20120142)*	1.0×10^5	61/67	91	47 vanB, 12 vanB+C, 1 vanA/B, 1 NS	26/30	87	35/37	95
VRE17-02	*E. faecalis* (equivalent to ATCC51299)	1.0×10^4	57/66	86	56 vanB, 1 vanA+B	26/31	84	31/35	89
VRE17-07	*E. faecalis* (equivalent to ATCC51299)	1.0×10^6	64/66	97	57 vanB, 5 vanA+B, 2 vanA/B	30/31	97	34/35	97
VRE17-08	*E. faecium* (IOWA2)	1.0×10^4	64/66	97	63 vanB, 1vanA/B	29/31	94	35/35	100
VRE17-10	*E. faecalis* (equivalent to ATCC51299)	1.0×10^5	62/66	94	59 vanB, 1 vanA+B, 2 vanA/B	28/31	90	34/35	97
VRE17-12	*E. gallinarum* (ENT20120142)*	1.0×10^5	60/66	91	49 vanB, 7 vanB+C, 1 vanC, 1 vanA+B, 2 vanA/B	28/31	90	32/35	91
VRE18-01	*E. faecalis* (equivalent to ATCC51299)	1.0×10^5	65/70	93	65 vanB	34/35	97	31/35	89
VRE18-03	*E. faecium* (IOWA2)	1.0×10^4	65/70	93	64 vanB, 1 vanA+B	34/35	97	31/35	89

VRE18-04	*E. faecalis* (equivalent to ATCC51299)	1.0 x 10^6	65/70	93	59 vanB, 4 vanA+B, 1 vanB+C, 1 NS	34/35	97	31/35	89
VRE18-06	*E. gallinarum* (ENT20120142)*	1.0 x 10^5	66/70	94	55 vanB, 9 vanB+C, 2 vanC	33/35	94	33/35	94
VRE18-11	*E. faecalis* (equivalent to ATCC51299)	1.0 x 10^4	64/70	91	64 vanB	34/35	97	30/35	86
	TOTAL		**1508/1640**	**91.7**		**622/675**	**91.6**	**886/965**	**91.7**

* *vanB* and *vanC* positive

NS: not specified

Table 5: Qualitative results for all *vanC* positive samples in the 6 years of VRE proficiency testing

Code	Content	Concentration (CFU/ml)	All datasets		Assays targeting vanC			Assays not targeting vanC		
			n	%	n	%	genes	n	%	genes
VRE13-06	*E. faecium* (ENT20130036) + *E. gallinarum* (LMG16289)	1.0×10^5 1.0×10^5	14/39	36	8/9	89	8 vanC	6/30	20	5 vanA, 1 vanA/B
VRE13-09	*E. gallinarum* (ENT20120142)*	1.0×10^6	34/39	87	9/9	100	1 vanB, 8 vanB+C	25/30	83	24 vanB, 1 NR
VRE14-04	*E. gallinarum* (ENT20120142)*	1.0×10^5	36/37	97	8/8	100	8 vanB+C	28/29	97	28 vanB
VRE14-06	*E. faecium* (MI12043391) + *E. gallinarum* (LMG16289)	1.0×10^5 1.0×10^5	8/37	22	6/8	75	5 vanC, 1 van A	2/29	7	1 van A, 1 non-vanA/B
VRE15-03	*E. gallinarum* (ENT20120142)*	1.0×10^5	47/49	96	9/10	90	8 vanB+C, 1 vanC	38/39	97	38 vanB
VRE15-11	*E. faecium* (MI12043391) + *E. gallinarum* (LMG16289)	1.0×10^5 1.0×10^5	7/49	14	7/10	70	7 vanC	0/39	0	/
VRE16-04	*E. faecium* (MI12043391) + *E. gallinarum* (LMG16289)	1.0×10^5 1.0×10^5	12/67	18	10/12	83	10 vanC	2/55	4	2 vanA
VRE16-12	*E. gallinarum* (ENT20120142)*	1.0×10^5	61/67	91	12/12	100	12 vanB+C	49/55	89	47 vanB, 1 vanA/B, 1 NS
VRE17-05	*E. faecium* (MI12043391) + *E. gallinarum* (LMG16289)	1.0×10^5 1.0×10^5	8/66	12	5/8	63	5 vanC	3/58	5	1 vanA, 1 vanB, 1 vanA+B
VRE17-12	*E. gallinarum* (ENT20120142)*	1.0×10^5	60/66	91	8/8	100	7 vanB+C, 1 vanC	52/58	90	49 vanB, 1 vanA+B, 2 vanA/B
VRE18-02	*E. faecium* (MI12043391) + *E. gallinarum* (LMG16289)	1.0×10^5 1.0×10^5	8/70	11	6/11	55	6 vanC	2/59	3	1 vanA, 1 vanB
VRE18-06	*E. gallinarum* (ENT20120142)*	1.0×10^5	66/70	94	11/11	100	9 vanB+C, 2 vanC	55/59	93	55 vanB
	TOTAL		**361/656**	**55.0**	**98/116**	**84.8**		**263/540**	**48.7**	

* *vanB* and *vanC* positive

NS: not specified

289 Table 6: False positive results for all *vanA/B/C* negative samples in the 6 years of VRE proficiency testing

Code	Content	Concentration (CFU/ml)	All datasets		Commercial assays		In-house assays	
			n	%	n	%	n	%
VRE13-03	*E. faecalis* (ENT20130032)	1.0×10^7	1/39	2.6	1/10	10.0	0/29	0.0
VRE13-04	*S. aureus* (ATCC25923)	1.0×10^6	2/39	5.1	0/10	0.0	2/29	6.9
VRE13-07	*E. faecium* (ENT20130036)	1.0×10^7	20/39	51.3	2/10	20.0	18/29	62.1
VRE13-12	negative (sample matrix)	-	3/39	7.7	1/10	10.0	2/29	6.9
VRE14-03	*E. faecalis* (ENT20130032)	1.0×10^6	0/37	0.0	0/11	0.0	0/26	0.0
VRE14-05	*S. aureus* (equivalent to ATCC25923)	1.0×10^5	1/37	2.7	0/11	0.0	1/26	3.9
VRE14-08	negative (sample matrix)	-	2/37	5.4	0/11	0.0	2/26	7.7
VRE14-14	*E. faecium* (MI12043391)	1.0×10^6	0/37	0.0	0/11	0.0	0/26	0.0
VRE15-05	*S. aureus* (equivalent to ATCC25923)	1.0×10^5	1/49	2.0	0/18	0.0	1/31	3.2
VRE15-06	*E. faecalis* (ENT20130032)	1.0×10^6	1/49	2.0	0/18	0.0	1/31	3.2
VRE15-09	negative (sample matrix)	-	0/49	0.0	0/18	0.0	0/31	0.0
VRE16-05	*E. faecalis* (ENT20130032)	1.0×10^6	2/67	3.0	1/30	3.3	1/37	2.7
VRE16-06	*S. aureus* (equivalent to ATCC25923)	1.0×10^5	0/67	0.0	0/30	0.0	0/37	0.0
VRE16-08	negative (sample matrix)	-	1/67	1.5	1/30	3.3	0/37	0.0
VRE17-03	*S. aureus* (equivalent to ATCC25923)	1.0×10^5	1/66	1.5	0/31	0.0	1/35	2.9
VRE17-04	*E. faecalis* (ENT20130032)	1.0×10^6	1/66	1.5	0/31	0.0	1/35	2.9
VRE17-09	negative (sample matrix)	-	0/66	0.0	0/31	0.0	0/35	0.0
VRE18-05	*E. faecalis* (ENT20130032)	1.0×10^6	0/70	0.0	0/35	0.0	0/35	0.0
VRE18-09	negative (sample matrix)	-	1/70	1.4	1/35	2.9	0/35	0.0
VRE18-10	*S. aureus* (equivalent to ATCC25923)	1.0×10^5	0/70	0.0	0/35	0.0	0/35	0.0

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Fig. 1: Detection rates of *vanA* (A) and *vanB* (B) positive samples over the years. (Error bars indicate the 95% confidence intervals, * p<0.05)