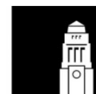

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1 Evaluation of the Novel artus C. difficile QS-RGQ, VanR QS-RGQ and MRSA/SA QS-
2 RGQ Assays for the Laboratory Diagnosis of Clostridium difficile Infection (CDI), and for
3 Vancomycin-Resistant Enterococci (VRE) and Methicillin Resistant Staphylococcus
4 aureus (MRSA) Screening.

5

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13

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15

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18 thank Cepheid for supplying the Xpert SA/Nasal Complete assays and technical advice.

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20

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ABSTRACT

Purpose: Clostridium difficile, methicillin resistant Staphylococcus aureus (MRSA) and vancomycin resistant enterococci (VRE) are worldwide prevalent healthcare associated pathogens. We have evaluated three Qiagen artus QS-RGQ assays for the detection of these pathogens.

Methods: We examined 200 stool samples previously tested for C. difficile infection, 94 rectal swabs previously screened for VRE, and 200 MRSA screening nasal swabs.

Results: With the routine diagnostic laboratory results being adopted as the gold standard, the sensitivity, specificity, PPVs and NPVs of the artus C. difficile assay were 100%, for the artus VanR QS-RGQ assay, 95%, 68%, 44% and 98%, and for the MRSA/SA artus assay, 80%, 94%, 93% and 83%, respectively. The artus VanR assay detected the vanA and/or vanB gene in 32% of culture-negative VRE screens, in 71% of these cases only vanB was detected. An over-estimation of the rate of faecal VRE colonisation could be due to a patient population with high rates of faecal carriage of non-enterococcal species carrying vanB.

Conclusions: Based on our findings we conclude that all three artus QS-RGQ assays could be a useful addition to a diagnostic laboratory, and that optimal choice of assay should be determined according to user needs.

Keywords: Healthcare acquired infections; MRSA; C. difficile; Vancomycin Resistant Enterococci; NAAT

INTRODUCTION:

Healthcare-associated infections (HCAIs) are a major cause of morbidity and mortality. [1-3] HCAIs can affect patients in any type of setting where they receive care and represent the most frequent adverse event in health care delivery worldwide. [4] Recent systematic reviews have estimated hospital-wide prevalence of HCAIs in high-income countries at 7.6% and in low and middle-income countries at 10.1%. [4] Clostridium difficile, methicillin resistant Staphylococcus aureus (MRSA) and vancomycin resistant enterococci (VRE) are three HCAI pathogens that are particularly prevalent worldwide.

Clostridium difficile infection (CDI) is considered the most common cause of nosocomial infectious diarrhoea among adults in the developed world. [5] The infection is related to antibiotic use and is associated with the overgrowth of *C. difficile* and the production of toxins A and/or B. These toxins cause a range of effects including mild to severe diarrhoea, gut mucosal damage, colitis, and pseudomembranous colitis. Recent figures report CDI as annually causing 1600 deaths in England and Wales, and 29000 deaths in the USA. [6,7] Since the clinical features of health care-associated diarrhea cannot reliably distinguish C. difficile from other causes, laboratory confirmation is essential. UK & European guidelines on the diagnosis of CDI recommend glutamate dehydrogenase (GDH) EIA or nucleic acid amplification testing (NAAT) to screen samples, followed by a sensitive toxin detection method. [8]

69 Vancomycin-resistant enterococci (VRE) are now amongst the most common HCAI
70 multidrug-resistant organisms. [9,10] Risk factors for nosocomial transmission of VRE
71 include prolonged hospitalization, use of broad spectrum antimicrobials and prior
72 surgery. VRE cause a range of infections including bloodstream, intra-abdominal,
73 surgical-site and urinary tract infections. [9, 11] Altogether, eight types of acquired
74 vancomycin resistance genotypes are known in enterococci with vanA being the most
75 prevalent genotype worldwide followed by vanB. [12, 13] Phenotypically the vanA gene
76 mediates a high-level of resistance to vancomycin and teicoplanin whereas the vanB
77 gene confers low- to moderate-level resistance to vancomycin only. Low level
78 vancomycin resistance expression, especially in vanB strains, may complicate
79 performance of diagnostic assays assessing the resistance phenotype and predicting
80 the corresponding genotype. During recent years, clusters of infections and
81 colonisations with vanB genotype E. faecium increased in a number of European
82 countries. [12]
83
84 MRSA is an important cause of HCAIs and community-acquired infections. [14, 15]
85 Patients colonized with MRSA serve as reservoirs for auto-infection and/or
86 dissemination to other patients and healthcare workers. [16, 17] Conventional
87 screening of MRSA is performed using selective and differential agar media, but the
88 results are not available before 18-48 hrs and interpretation can be subjective. Faster
89 detection can be achieved by using PCR-based assays. There is ongoing debate
90 regarding which tests are more appropriate for screening programmes. The increased

91 cost of rapid tests may be offset by savings as a result of reduced cross infection, fewer
92 complications, and better utilization of beds. [18, 19]
93 Rapid and accurate detection of CDI, MRSA and VRE is required to ensure patients
94 receive appropriate antimicrobial treatment and optimised infection prevention
95 interventions. QIAGEN has developed real-time, multiplex, PCR assays for detection of
96 these three HCAI pathogens. The *artus* C. difficile QS-RGQ assay (CE marked and FDA
97 cleared) detects the *tcdA* and *tcdB* genes that encode for *C. difficile* toxin A and toxin B,
98 respectively; the *artus* VanR QS-RGQ (CE marked) assay detects the *vanA* and *vanB*
99 genes of enterococci; the artus MRSA/SA QS-RGQ assay (CE marked) detects the
100 lhd1, mecA and mecC genes of MRSA. We have evaluated the performance of these
101 three molecular assays in comparison with conventional testing methods.
102
103 **MATERIALS AND METHODS:**
104 We compared the performance of the QIAGEN *artus* assays (QIAGEN, GmbH, Hilden,
105 Germany) with the routine identification methods at the Leeds Teaching Hospitals NHS
106 Trust (LTHT) Microbiology Dept. The *artus* assays were performed on the automated
107 QIAsymphony RGQ system (QIAGEN). All samples tested were selected from those
108 submitted to the routine laboratories.
109
110 **Qiagen artus C. difficile assay**
111 A total of 200 stool samples received by the routine enteric laboratory between
112 December 2013 and May 2014 from patients aged ≥ 2 years was selected for inclusion
113 in the study. All samples were diarrheal (adopting the shape of the container), had been

114 submitted for glutamate dehydrogenase (GDH) detection and cytotoxin testing (CTT),
115 and had sufficient material to allow for all testing required. The study set comprised 100
116 specimens previously found to be positive for both GDH antigen and cytotoxin
117 production, and 100 GDH-negative specimens. Samples previously GDH and cytotoxin
118 positive were between one day and four months old at the time of testing, negative
119 samples were processed within one week of collection. All samples were stored at 2-
120 5℃ prior to testing. S amples were processed with the *artus* C. difficile QS-RGQ assay
121 according to manufacturer's instructions. Previously GDH/CTT-positive stool samples
122 were re-analyzed for the GDH antigen using the C diff Chek-60 glutamate GDH assay
123 (Techlab, Blacksburg, VA, USA) according to manufacturer's instructions and also for
124 the presence of cytotoxin. In brief, stool samples were first diluted 1:5 in phosphate-
125 buffered saline before being centrifuged, 20 µl of supernatant were then added to
126 duplicate Vero cell monolayers. One these had been protected by the addition of 20 µl
127 Clostridium sordelli antitoxin (Prolab Diagnostics, UK). Vero cells were grown in 96-well
128 flat-bottomed microtitre trays in 160 µl of Dulbecco medium. A positive result was
129 recorded if cell rounding was observed in the unprotected cells only, after 24 or 48
130 hours of incubation at 37°C in the presence of CO_2.
131

132 **Qiagen *artus* VanR QS-RGQ assay**

133 Twenty rectal swabs positive for VRE, as determined by culture on Kanamycin Aesculin
134 Azide (KAA) agar plus vancomycin (E&O Laboratories, Bonnybridge, Scotland) and
135 subsequent MALDI-TOF analysis, were collected from inpatients during April and May
136 2014. A further 74 VRE culture-negative rectal swabs collected during this period were

137 also examined. Transport swabs containing Aimes Medium with charcoal were used
138 (Thermo Fisher Scientific, Loughborough, UK). All samples were stored at 2-5°C prior to
139 analysis and were processed using the *artus* VanR QS-RGQ assay as per
140 manufacturer's instructions.
141
142 **Qiagen *artus* MRSA QS-RGQ assay**
143 In total, 200 nasal swabs processed by the MRSA screening laboratory between
144 January and June 2014 were retrospectively selected for inclusion in the study.
145 Transport swabs containing Aimes Medium with charcoal were used (Thermo Fisher
146 Scientific, Loughborough, UK). One hundred of these samples had previously been
147 determined MRSA positive and 100 MRSA negative, by culture on Brilliance MRSA 2
148 Agar (Oxoid, Basingstoke, UK). All samples were stored at 2-5°C prior to analysis. The
149 MRSA-positive samples were between three weeks and five months old at the time of
150 testing; all negative samples were processed within one week. Previously MRSA-
151 positive samples were first inoculated onto Brilliance MRSA 2 culture medium and
152 subsequently processed using the *artus* MRSA QS-RGQ assay. Previously MRSA-
153 negative swabs were processed with the artus assay but were not inoculated onto the
154 culture medium. Sample preparation for the artus assay involved placing the swabs into
155 tubes containing 2.5 ml eNat medium (Copan, Brescia, Italy) followed by vigorous
156 swirling. The eNat tubes were then placed directly onto the QIAsymphony instrument
157 and the assay performed according to the manufacturer's instructions. Residual sample
158 volumes within the eNat tubes following analyses were processed using the Xpert

159 SA/Nasal Complete assay (Cepheid, Sunnyvale, CA, USA) on the GeneXpert

160 automated platform according to manufacturer's instructions.

161

162 **RESULTS**

163

164 **Qiagen artus C. difficile assay**

165 The sensitivity, specificity, PPVs and NPVs of the artus C. difficile assay were all 100%

166 (CI 95-100%) when the original results of the diagnostic laboratory algorithm

167 (GDH/CTT) were adopted as the gold standard. Of the 100 GDH/CTT-positive samples

168 re-tested against this algorithm, 96% remained both GDH and CTT positive. A

169 breakdown of the discrepant results is displayed in Table I.

170

171 **Qiagen *artus* VanR QS-RGQ assay**

172 The sensitivity, specificity, PPVs and NPVs of the artus VanR QS-RGQ assay were

173 95% (19/20, 95% CI 73-100%), 68% (50/74, 95% CI 56-78%), 44% (19/43, 95% CI 29-

174 60) and 98% (50/51, 95% CI 88-100%) respectively where direct culture followed by

175 MALDI-TOF analysis was adopted as the gold standard. The assay detected the vanA

176 and/or vanB gene in 32% of culture negative VRE screens. In 71% of these cases

177 (n=17), only vanB was detected; in 25% of cases (n=6), both vanA and vanB were

178 detected with vanA alone being detected in the remaining 4% (n=1).

179

180 **Qiagen artus MRSA QS-RGQ assay**

181 Of the 200 nasal swabs previously analysed for presence of MRSA by culture, eight
182 MRSA-positive and 32 MRSA-negative swabs gave either an invalid result (n = 19) or
183 an error message (n = 21) with the Xpert SA Nasal Complete assay. The majority of the
184 errors (95%) were due to probe check failures. This was most likely due to the off-label
185 nature of the methodology, specifically the charcoal content of the swab transport
186 medium. These samples were eliminated from the study and results from the remaining
187 160 nasal swabs analysed. Adopting the original culture results as the gold standard,
188 the sensitivity, specificity, PPV and NPV values for the artus assay were 64% (58/91,
189 95% CI 53-73%), 94% (64/68, 95% CI 85-98%), 94% (58/62, 95% CI 84-98) and 66%
190 (64/97, 95% CI 56-75) respectively, and for the Xpert assay, 74% (67/91, 95% CI 63-
191 82), 97% (66/68, 95% CI 89-99, 97% (67/69, 95% CI 89-99) and 73% (66/90, 95% CI
192 72-90). The distribution of results obtained from both PCR assays is displayed in Table
193 II. A total of 31 (19.4%) samples gave discordant results. The majority of discrepancies
194 were results which were interpreted by the artus assay as S. aureus, but designated
195 'not detected' by the Xpert assay (39%), followed by results interpreted as MRSA by the
196 Xpert assay, but designated S. aureus by the artus assay (32%). The majority of the
197 results from this latter group (9/10) were found to be MRSA positive by culture.
198
199 Of the 92 MRSA-positive swabs, only 71% were positive upon re-culture. There was
200 good correlation between samples which were MRSA-positive upon re-culture, and
201 those samples which were MRSA positive with one or both PCR assays. A second set
202 of statistics was therefore calculated following elimination of results from all swabs that
203 were MRSA-negative upon re-culturing; the sensitivity, specificity, PPV and NPV values

204 for the artus assay were then 80% (52/65, 95% CI 68-89), 94% (64/68, 95% CI 85-98),
205 93% (52/56, 95% CI 82-98) and 83% (64/77, 95% CI 72-90), and for the Xpert assay,
206 88% (57/65, 95% CI 77-94%), 97% (66/68, 95% CI 89-99), 97% (57/59, 95% CI 87-99)
207 and 89% (66/74, 95% CI 79-95), respectively.

208 **DISCUSSION**

209

210 There is still conflicting opinion on optimal diagnosis of CDI worldwide [20-23].
211 Inaccurate laboratory results may lead to unnecessary treatment and isolation, and the
212 true cause of the patients' diarrhoea not being further investigated (in the case of false
213 positives), or cross-infection may occur with other patients and overtreatment with
214 empirical antibiotics (in false-negative cases). A GDH test or NAAT are recommended
215 for initial screening of samples because of their very high sensitivities, reported to be
216 79.5-100% [8, 23, 24], followed by a sensitive toxin detection assay.

217

218 In this study the performance of the artus C. difficile QS-RGQ assay was equivalent to
219 that of a recommended two-step algorithm when testing samples retrospectively. A
220 limitation of the study was that GDH-positive/CTT-negative samples were not included
221 within the evaluation. However, although the sample selection is not necessarily
222 representative of that seen in a routine hospital setting due to the proportion of positive
223 samples being preselected, this is a useful evaluation of assay performance for the
224 detection of true CDI positives and true CDI negatives. It is possible that excluding
225 GDH-positive/CTT-negative samples may have slightly improved the performance of the
226 artus C. difficile assay, however, this comparison most likely gives a more meaningful

227 result in the context of CDI diagnosis as such results do not represent CDI, but possible
228 colonisation.
229
230 It was noted that in previously CTT-positive samples that were toxin-negative upon re-
231 testing, it had either taken 48 hrs for a cytotoxic effect to occur in the initial test, or this
232 effect had only been observed with an undiluted sample. This suggests that there were
233 low levels of toxin in these samples. It is possible that toxin degradation during storage
234 may have lowered the concentration further to an undetectable level. As PCR assays
235 detect the presence of the toxin genes as opposed to the presence of free toxin, the
236 artus assay has potential to identify faecal samples as toxin gene positive when they
237 contain low toxin levels that are undetectable by CTT. Studies by Jazmati et al (2015)
238 and Moon (2016) found the performance of the artus C. difficile to be comparable to that
239 of the Xpert C. difficile PCR assay. [25, 26] It must be considered that a proportion of
240 hospitalised patients may have toxigenic C. difficile with asymptomatic carriage and
241 diarrhoea due to another cause. However, although a number of publications
242 recommend that NAATs should not be used alone to diagnose CDI, [8, 24, 27, 28] some
243 studies have found good correlation between toxin gene detection and clinical status of
244 the patient. [29, 30]
245
246 As the artus C. difficile assay (as with the further two artus assays evaluated) has a
247 short turnaround time (3h 40 min for 24 samples which includes approximately 30-40
248 mins hands on time), is user friendly, includes simple interpretation of results, has a
249 high throughput (up to 72 per run) and offers flexibility of the associated platform for

250 detection of other organisms, this assay is a useful addition to the detection of CDI. As
251 there is still no internationally accepted single method for CDI diagnosis, individual
252 laboratories must decide which test will integrate best into their existing workflow. Two-
253 or three-step approaches to the diagnosis of CDI could increase laboratory costs, but
254 these might be offset by reduced total health care costs.

255

256 **Vancomycin-resistant enterococci**

257 As with CDIs, rapid and accurate detection of VRE is essential for adequate patient
258 management including infection prevention measures. Traditional culture-based
259 methods to detect these organisms are often time-consuming, taking up to several days
260 to complete. A number of NAAT assays are now available that can detect either the
261 vanA gene, or both vanA and vanB genes. Assays detecting both of these genes are
262 desirable as a number of European countries have reported increasing numbers of
263 colonisations and infections with vanB-type VRE. [12, 31]

264

265 The sensitivity of the artus vanR assay in our study (95%) compares favourably with
266 NAATs in previous studies [32]. Although the assay specificity was low (44%), it is
267 possible that the false-positives recorded actually represented genuine VRE positive
268 samples, where bacterial growth was not supported by the culture medium. High rates
269 of vanB carriage have previously been reported in the absence of cultivable VRE in
270 fecal/rectal samples and have mostly been attributed to one of two explanations. The
271 first is that vanB-type resistance is sometimes difficult to detect since the vancomycin
272 MIC of these strains can be below the antimicrobial susceptibility breakpoint of ≤4

273 mg/liter defined by the European Committee on Antimicrobial Susceptibility Testing.
274 (EUCAST) [31, 33, 34] Secondly, non-enterococcal vanB genes could results in positive
275 PCR results. These can be found in the gut, especially in anaerobic bacteria like
276 Clostridium species. [32, 35-37] In our study the vanB gene alone was detected in 71%
277 of these false-positive samples; our culture medium contained 6 mg/l vancomycin, and
278 so either of these explanations is feasible. The artus VanR QS-RGQ assay may indeed
279 be more sensitive for the detection of VRE isolates than culture due to the amplification
280 of both vanA and vanB genes. However, further investigations on vanB positive (but
281 vanA negative) isolates are needed to further knowledge here. If a patient population
282 has high rates of faecal carriage of non-enterococcal species that contain vanB, an
283 over-estimation of the rate of faecal VRE colonisation could result and potentially lead to
284 unnecessary utilization of hospital resources and infection control prevention measures.
285

286 **MRSA**

287 Rapid and accurate detection of MRSA is required to minimize the spread of this
288 organism in healthcare settings. Active screening currently forms an integral part of
289 many MRSA infection control and prevention strategies, with several NAAT assays
290 available for this purpose.
291

292 Although the sensitivity values and NPVs of the artus and Xpert assays were low when
293 calculated using the whole sample set, these values were much improved when the
294 33% of swabs originally MRSA culture-positive, but negative upon re-culture, were
295 eliminated from the calculations. It is likely that bacterial degradation occurred during

296 storage, and so these improved figures are more likely representative of assay

297 performance during prospective testing. Our study was performed using an off-label

298 method that incorporated charcoal-containing transport swabs. The invalid samples and

299 the error messages from the Xpert assay were presumed to be due to interfering or

300 inhibiting factors within the charcoal. The sensitivity of the Xpert assay (88%) is

301 comparable to those of other MRSA PCR assays (82-93%), although the sensitivity of

302 the artus assay was slightly lower at 80%.

303

304 Specificities of both assays were relatively high (94% and 97% for the artus and Xpert

305 assay, respectively) and compare well with other studies (78-99%). [38-42] In most

306 cases (80.5%-90.5%), the two assays were in agreement as to whether MRSA, MSSA

307 or no targets were present. The most common discordant combination was MSSA

308 detected by the artus assay but no targets detected by the Xpert assay (12/160).

309 However, in an MRSA screening programme, this would not affect patient management.

310 The second most common discordant category was where the artus assay detected

311 MSSA but the Xpert assay detected MRSA (10/160). Such results would affect patient

312 management and are therefore of more concern. Almost all (9/10) of these samples

313 were MRSA positive by culture, which suggests that the artus assay misidentified these

314 samples. The Xpert assay, unlike the artus assay, does not detect mecC variants;

315 however, only 2.6% of samples were designated MRSA by the artus assay only, and

316 3/4 of these were culture-negative. This study therefore does not highlight lack of

317 detection of mecC variants as a significant issue. Although the Xpert assay is rapid and

318 simple to use when processing smaller sample volumes, the artus assay is more
319 efficient when processing larger volumes and is less costly per test**.**
320
321 As with the artus C. difficile and VanR assays, the optimal choice of assay for MRSA
322 screening should be determined according to user needs; for example, the artus MRSA
323 assay would be more suited to a laboratory handling high volumes of screening swabs.
324 The three artus assays all have high NPVs and are therefore especially suited to
325 screening programmes. The assays can facilitate elimination of negative samples,
326 meaning that confirmatory tests are only needed on a small proportion of these. This
327 could reduce the hands on time required overall and lead to negative results being
328 released more quickly. Additional laboratory-specific factors, including financial
329 considerations and technical expertise, will also be important in deciding between
330 screening methods.
331
332 **COMPLIANCE WITH ETHICAL STANDARDS**
333
334 **Funding**: This study was funded by Qiagen (grant number 403774_Qiagen_2014)
335 **Conflict of interest:** MHW declares that he has the following conflict of interests: I have
336 received consulting fees from Qiagen and grant funding from Cepheid. In addition,
337 QIAGEN supplied the QIAsymphony RGQ MDx system, artus assays, related reagents
338 and consumables, as well as technical expertise. Cepheid supplied the Xpert SA/Nasal
339 Complete assays and technical advice.
340
341 **Ethical approval**: No ethical approval was required for this study.

342

343 **Informed consent**: No informed consent was required for this study.

344

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544 **Table I: Discrepant GDH/CTT algorithm results**

Result of repeat algorithm testing		No. of samples
GDH	CTT	
-	+	1
+	-	2
-	-	1

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546 **Table II: Distribution of results obtained from the artus MRSA and GeneXpert**

547 **SA/Nasal assays**

artus MRSA result	Xpert SA/Nasal result	No. of samples (%)	No. of samples culture positive (%[a])
MRSA	MRSA	58 (36.3)	57 (98)
MRSA	MSSA	3 (2.0)	0 (0)
MRSA	No targets detected	1 (0.6)	1 (100)
MSSA	MRSA	10 (6.3)	9 (90)
MSSA	MSSA	18 (11.3)	3 (16.7)
MSSA	No targets detected	12 (7.5)	5 (41.7)
No targets detected	MRSA	1 (0.6)	1 (100)
No targets detected	MSSA	4 (2.5)	1 (25)
No targets detected	No targets detected	53 (33.0)	15 (28)

548 [a] denominator being the number of samples within each individual category