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MRSA Screening: Can one swab be used for both culture and rapid testing? An evaluation of chromogenic culture and subsequent Hain GenoQuick® PCR amplification/detection.

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MRSA Screening: Can one swab be used for both culture and rapid testing?
An evaluation of chromogenic culture and subsequent Hain GenoQuick® PCR amplification/detection.

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Abstract:	<p>We evaluated the Hain GenoQuick® (GQM) methicillin resistant <i>Staphylococcus aureus</i> (MRSA) assay for the rapid detection of MRSA using one swab, i.e. the same screening specimen was used first for MRSA culture and then for rapid testing by PCR, as this would be the preferred option for routine diagnostic testing. GQM detected current prevalent Irish MRSA strains incorporating all known SSCmec types including Panton-Valentine leukocidin positive strains. All methicillin resistant coagulase negative staphylococci tested were negative but three of seven gentamicin-resistant MSSA strains tested were identified as MRSA by the GQM method. The theoretical ex-vivo limit of detection of the assay was 704 colony forming units (CFU) per GQM assay reaction (1.7×10^4 CFU/ml) when MRSA suspensions were used for DNA extraction or 1.4×10^3 CFU/swab (1.4×10^4 CFU/ml) using MRSA absorbed onto Copan screening swabs. We demonstrated that swab processing on chromogenic agar prior to PCR resulted in some inhibition of the PCR reaction, increasing the limit of detection of the assay by a factor of 4. Based on the processing of 540 screening specimens (nasal and groin) by culture first and GQM second, the specificity and positive predictive value were both 100%, the negative predictive value was 92%, and the sensitivity was 57%. Culture followed by PCR from one specimen is not optimal for the rapid detection of MRSA. Further laboratory validation of the GQM assay is required to determine the true diagnostic sensitivity and value of this kit in routine microbiology laboratories, either with PCR before culture or using two specimens.</p>

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For Peer Review

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3 1 **MRSA Screening: Can one swab be used for both culture and rapid testing?**
4
5 2 **An evaluation of chromogenic culture and subsequent Hain GenoQuick® PCR**
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7 3 **amplification/detection.**
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9 4

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Abstract

We evaluated the Hain GenoQuick® (GQM) methicillin resistant *Staphylococcus aureus* (MRSA) assay for the rapid detection of MRSA using one swab, i.e. the same screening specimen was used first for MRSA culture and then for rapid testing by PCR, as this would be the preferred option for routine diagnostic testing. GQM detected current prevalent Irish MRSA strains incorporating all known *SSCmec* types including Pantone-Valentine leukocidin positive strains. All methicillin resistant coagulase negative staphylococci tested were negative but three of seven gentamicin-resistant MSSA strains tested were identified as MRSA by the GQM method.

The theoretical ex-vivo limit of detection of the assay was 704 colony forming units (CFU) per GQM assay reaction (1.7×10^4 CFU/ml) when MRSA suspensions were used for DNA extraction or 1.4×10^3 CFU/swab (1.4×10^4 CFU/ml) using MRSA absorbed onto Copan screening swabs. We demonstrated that swab processing on chromogenic agar prior to PCR resulted in some inhibition of the PCR reaction, increasing the limit of detection of the assay by a factor of 4. Based on the processing of 540 screening specimens (nasal and groin) by culture first and GQM second, the specificity and positive predictive value were both 100%, the negative predictive value was 92%, and the sensitivity was 57%. Culture followed by PCR from one specimen is not optimal for the rapid detection of MRSA. Further laboratory validation of the GQM assay is required to determine the true diagnostic sensitivity and value of this kit in routine microbiology laboratories, either with PCR before culture or using two specimens.

47 Introduction

48 A major component of the control of methicillin-resistant *Staphylococcus aureus*
49 (MRSA) transmission is the early detection of patients either colonised or infected with
50 MRSA, followed by isolation to prevent cross-infection [1]. Several publications
51 evaluating the accuracy of various MRSA detection kits (IDI MRSA, GeneXpert MRSA,
52 GenoType® MRSA and the MRSA EVIGENETM) and PCR in-house assays [2-8] have
53 been published. The Hain GenoQuick® (GQM) methicillin resistant *Staphylococcus*
54 *aureus* (MRSA) assay (Hain Lifescience, Nehren, Germany) is a new molecular assay. Its
55 predecessor GenoType® MRSA is a PCR assay using DNA Strip® technology for
56 amplicon detection, and permits the identification of *S. aureus*, *S. epidermidis*, and the
57 *mecA* gene, with a specimen turn around time of approximately seven hours [7, 9,]. GQM
58 is based on the same immuno-chromatographic detection technology, but detects MRSA
59 only with a reported turn around time of approximately two and a half hours. MRSA
60 specific chromosomal sequences are targeted. The assay consists of three steps: direct
61 DNA extraction from a patient swab using a lysis buffer provided, followed by multiplex
62 PCR (primer/probe/nucleotide mix provided) with single stranded amplicon hybridization
63 using a specific probe and finally, the detection of amplicon/probe complex - the complex
64 selectively binds to the test band on the dipstick and is visualized by gold labeling. Each
65 dipstick includes two control zones, a conjugate control zone to check the binding of the
66 conjugate on the dipstick and an amplification control zone to check for a successful
67 amplification reaction.

68 Here we determined the accuracy and the limit of detection (LoD) of the GQM
69 assay and established the potential to use one MRSA screening swab for two methods of

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3 70 MRSA detection. This specimen process flow is preferred where diagnostic laboratories
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5 71 choose to continue culture with PCR, for confirmation and to use the isolate for
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7 72 epidemiological purposes. Additionally, this specimen flow enabled adherence to
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9 73 accredited laboratory standard operating procedures, minimised the additional
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11 74 nursing/laboratory and administrative workloads, and the financial burden associated
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13 75 with the taking and processing of two specimens.

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17 **Methods**18 **Bacterial isolates**

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21 79 The capacity of the GQM assay to detect Irish MRSA strains (n=32) was assessed
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23 80 with a collection provided by the National MRSA Reference Laboratory, Dublin and
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25 81 Dublin Dental School and Hospital, Trinity College Dublin [10,11]. All isolates were
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27 82 tested according to manufacturer's instructions for pure culture testing. This isolate group
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29 83 comprised of 27 isolates representative of common epidemiological types recovered in
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31 84 Ireland and four control strains, including the following SCCmec types (I, IA, II, IIA-IIE,
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33 85 III, IIIv, IV and IVa-IVh, V, VI). Two reference strains, MRSA-ATCC 43300 and
34
35 86 methicillin sensitive *Staphylococcus aureus* (MSSA)-ATCC 29213 were included. A
36
37 87 further seven gentamicin-resistant methicillin-susceptible *Staphylococcus aureus*
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39 88 (GrMSSA) strains [12] and eight various methicillin resistant coagulase negative
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41 89 staphylococci (MRCNS) were also tested.

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45 **Conventional culture and MRSA identification**

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47 92 MRSA screening specimens were collected using single Copan 151C cotton albumin
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49 93 coated swabs (Medical Supply Company, Ireland) as per laboratory standard operating

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94 | procedures. The GQM assay does not specify what type of swab is optimal for use.
95 | Swabs were assessed for the presence of MRSA by plating on MRSA Select@
96 | chromogenic media (CA) for the isolation and identification of MRSA (Bio-Rad Life
97 | Science Group, France). Colonies deemed positive on this media were subcultured on
98 | Columbia Blood Agar (CBA) (Cruinn, Ireland) and confirmed by slide coagulase
99 | (Staphaurex Plus, Remel, Oxoid Ltd., U.K.) and by automated antibiotic susceptibility
100 | testing (Phoenix, BD Biosciences, USA).

102 **Limit of detection (LoD) assays**

103 | LoD assays (n=3) of the GQM assay were performed as follows: A 0.5 M^cFarland
104 | of strain MRSA ATCC 43300 was prepared in saline and a 10-fold dilution series carried
105 | out. CFU/ml was determined by spread plating each dilution on CBA in and incubating at
106 | 37°C for 24 h.

107 | To calculate the LoD of the assay when MRSA suspensions were used directly for
108 | DNA extraction protocols, 40 µl of each dilution above was added to 260 µl of buffer Q-
109 | LYS supplied in the GQM kit. Subsequent extraction of these 300 µl preparations, PCR
110 | and ELISA based detection were performed as per the manufacturers' instructions.

111 | To calculate the LoD when MRSA suspensions were absorbed onto Copan
112 | screening swabs prior to DNA extraction, 100 µl of each dilution above was adsorbed
113 | onto an individual swab. The swab was allowed to dry for 30 min before vigorous
114 | vortexing in 300 µl of buffer Q-LYS and GQM processing.

115 | Using the CFU/ml count determined above, the LoD of each reaction was
116 | determined, and the equivalent CFU/ml was calculated. LoD were recorded as the lowest
117 | concentration which produced a GQM assay positive reaction.

Deleted: when cells were added directly to the PCR reaction, 4

Deleted: where cells were absorbed onto Copan screening swabs prior to PCR amplification,

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Chromogenic agar (CA) inhibition

119 GQM assay inhibition by CA agar

120 To establish if culturing screening specimens on CA prior to GQM processing
121 resulted in any PCR reaction inhibition the following experiment was carried out (n=3).

122 A 10-fold dilution series of a 0.5 M^cFarland *S. aureus* ATCC 43300 was made in 1ml
123 volumes of sterile saline. 40 µl of each dilution was added to 260 µl of GQM buffer Q-
124 LYS. A duplicate set of dilutions was prepared. In the first set, a sterile Copan swab was
125 rubbed on a CA plate just before washing in each 300 µl dilution and processing by
126 GQM. In the second set, a sterile Copan swab that had not been exposed to CA agar was
127 washed in each 300 µl dilution and processed by GQM. The number of CFU per dilution
128 was determined by spread plating (as for LoD above). LoD results were compared to
129 determine if rubbing the swab on CA prior to PCR had an inhibitory affect.

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131 **GQM version 2.0**

132 All tests were performed according to the manufacturer's instructions except that
133 specimens were cultured on CA prior to washing the swab in lysis buffer. The PCR
134 amplification mix consisted of 35 µl primer nucleotide mix, 5 µl 10x polymerase buffer,
135 0.5 µl of 25mM MgCl₂ 0.3 µl Fast start Taq (5u per µl) (Roche), 5 µl DNA template and
136 4.2 µl water. The amplification protocol was according to the manufacturers instructions.

137

138 **Patient specimen collection and processing**

139 Nasal and groin swabs were collected from patients on various hospital wards in a
140 700-bed tertiary referral hospital with endemic MRSA, over an eight week period as part
141 of routine MRSA screening measures. Five hundred and forty specimens from 250

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3 142 patients were collected and processed (270 groin and 270 nasal). Swabs were cultured on
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5 143 CA plates before processing by GQM. Discrepant results were further examined as
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7 144 follows. Swabs that were PCR positive, culture negative were cultured a second time, but
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9 145 were enriched overnight in 5 ml of tryptone salt broth 6% NaCl (37°C at 150 rpm in a
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11 146 shaking incubator) with subsequent plating on CA. Specimens that were PCR negative,
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13 147 culture positive were processed again by PCR i.e. as per manufacturer's instruction 5 µl
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15 148 of the 300 µl extracted specimens were tested a second time by GQM PCR. Additionally,
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17 149 the MRSA isolate recovered from the discrepant specimen was tested by PCR in pure
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19 150 culture as per manufacturer's instructions.

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22 152 **Results**

23 153 **Analytical sensitivity and specificity**

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27 154 The GQM assay detected all MRSA strains in a collection representative of
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29 155 MRSA in Ireland since 1974 (n=32). All eight MRCNS tested negative. Of seven
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31 156 GrMSSA strains tested, 3 tested positive by PCR and are considered false positives.

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34 35 158 **LoD assays**

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37 159 Using a saline dilution series and plate counting, the LoD when MRSA
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39 160 suspensions were used directly for DNA extraction and subsequent GQM amplification
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41 161 was determined to be 704 CFU/GQM reaction (equivalent to a calculated 1.7×10^4
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43 162 CFU/ml). As only 5 µl of the extracted 300 µl DNA preparations can be used as template
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45 163 in the GQM PCR reaction, it can be said the LoD per PCR is 11.8 CFU.

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3 164 The LoD when MRSA suspensions were absorbed onto Copan screening swabs
4 165 prior to DNA extraction and subsequent GQM amplification was determined to be
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6 166 1.4×10^3 CFU/swab equivalent to a calculated 1.4×10^4 CFU/ml.
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11 168 **Chromogenic agar inhibits the Hain® GQM assay**

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13 169 In a GQM reaction where a swab was washed in GQM buffer Q-LYS containing a
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15 170 known number of MRSA cells, the lowest number of MRSA detected was 1,390
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17 171 CFU/reaction. In contrast, where the swab was first rubbed on a CA plate and then
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19 172 washed in GQM buffer Q-LYS containing the same number of MRSA cells as above, the
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21 173 lowest number of MRSA detected was 5,560 CFU/reaction, suggesting processing of
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23 174 swabs on CA prior to GQM PCR does have an inhibitory effect and increases LoD per
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25 175 reaction or CFU/ml by a factor of 4 (approximately).
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29 177 **Clinical specimens**

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31 178 Of 540 specimens processed 47/540 (8.7%) were both PCR and culture positive
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33 179 and 451/540 (83.5%) were negative by both methods. Discrepant results occurred with
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35 180 42/540 (7.7%) specimens; 4 were PCR positive but culture negative and 38 were PCR
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37 181 negative but culture positive. All four patients with a positive PCR and culture negative
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39 182 result had been decolonised and thus were considered true MRSA positives. The
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41 183 amended MRSA positive rate was therefore calculated as 51/540 (9.4%), with a
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43 184 discrepant rate of 38/540 (7.0%). For these remaining 38 discrepant results (PCR
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45 185 negative/culture positive specimens), PCR rechecks of the prepared DNA extracted/lysis
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47 186 reactions were carried out and all were confirmed negative.
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3 187 All 38 swabs were plated a second time on CA and subsequently enriched in TSB
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5 188 broth for 24 h followed by plating on CA. Twelve grew after direct plating and
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7 189 enrichment, suggesting the microorganisms were present on the swabs but were not
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9 190 detected by PCR. PCR on all 12 of these isolates (colony tested) were PCR positive,
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11 191 therefore these were considered true PCR false negatives. *S. aureus* was not isolated
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13 192 again after repeat culture with enrichment in TSB for the remaining 26/38 discrepant
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15 193 specimens. These were considered false negatives. The sensitivity, specificity, positive
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17 194 predicative value (PPV) and negative predictive value (NPV) were calculated (Table 1).

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20 196 **Discussion**

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22 197 Conference proceedings by Eigner *et al* [13] report on an evaluation of the GQM
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24 198 assay and report this assay as 100% sensitive, with a specificity of 99.4%, PPV 96% and
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26 199 NPV 100% while Boegli-Stuber [14] report a GQM sensitivity of 91.3%. These results
27
28 200 were promising as GQM was 50% cheaper than other molecular methods, it could
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30 201 provide a cost effective alternative for rapid MRSA diagnostics. Additionally this study
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32 202 showed that GQM detects SCC*mec* types I-V, while MSSA or coagulase negative
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34 203 *Staphylococci* test negative. Our data supports this, with SCC*mec* VI also being detected.
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36 204 However, three of seven GrMSSA strains tested here produced a GQM positive, culture
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38 205 negative result. These strains [12] harbor remnants of SCC*mec* DNA. As the majority of
39
40 206 commercially available assays use PCR to detect SCC-associated sequences directly from
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42 207 clinical specimens, a PCR positive/culture negative results warrants caution and further
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44 208 investigation, e.g. culture on non selective media for isolate characterisation.

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3 209 The LoD of GQM can be reported in a number of ways. Here we established the
4 210 LoD of the complete GQM assay to be 704 CFU. This is the minimum number of MRSA
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6 211 cells required to be added to the GQM extraction mix that will result in a positive
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8 212 hybridization signal. LoD can also be expressed as LoD per PCR and has this has been
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10 213 calculated as 11.8 CFU per PCR. As many studies report LoD of rapid assays in CFU per
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12 214 ml or CFU per swab these GQM LoD results can also be reported as 1.4×10^4 CFU/ml or
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14 215 1.7×10^3 CFU/swab. By contrast, the LoD of other rapid methods have been reported as
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16 216 6.1×10^2 CFU/ml or 5.8×10^1 CFU/swab for GeneXpert and 1.7×10^2 CFU/swab for
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18 217 routine MRSA chromogenic culture [4].

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21 218 Processing clinical specimens on CA and subsequent GQM amplification as
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23 219 described here results in a discrepant rate of 7% (38 specimens were PCR negative,
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25 220 culture positive). Twelve of the 38 discrepant specimens reported as PCR negative/
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27 221 culture positive, were culture positive both prior to GQM processing and when cultured a
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29 222 second time after discharge in the Q-LYS lysis buffer, suggesting that these were false
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31 223 negatives by GQM.

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33 224 However, 26/38 specimens that were culture positive/PCR negative were not
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35 225 found to be culture positive after a second attempt to culture them following discharge in
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37 226 the GQM buffer Q-LYS solution. Some possible explanations for these discrepancies
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39 227 include: (i) discrepancy due to this workflow i.e. processing by culture before GQM
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41 228 removed all organisms from the swab hence a GQM negative result (ii) PCR inhibition
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43 229 resulted from rubbing the swab on a CA plate prior to GQM processing (iii) the lysis
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45 230 reaction may have failed or may not have been optimal but there is no control for this
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47 231 reaction when using the GQM kit and (iv) these 26 specimens were not detected as GQM

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3 232 positive because inoculum levels were below the LoD of the GQM assay. Depending on
4 233 the interpretation of these findings various sensitivities could be calculated. If we
5 234 excluded the 26 specimens from sensitivity calculations that were GQM negative, culture
6 235 positive from the analysis, assuming that because of the work flow used all organisms
7 236 had been removed by culture prior to PCR or CA inoculation resulted in inhibition, a
8 237 GQM sensitivity of 81% would be achieved. If we included the 26 specimens in the
9 238 sensitivity calculation but assumed that all 26 specimens would have been PCR
10 239 positive/culture positive if specimens had been processed by PCR first followed by
11 240 culture, then an improved sensitivity of 87% would result. However, the actual sensitivity
12 241 determined here was 57%. The true value for the sensitivity value is at best between
13 242 81%-87% but at worst between 57-81%, depending on the interpretation of the PCR
14 243 negative culture positive discrepant results. A full study to establish the diagnostic
15 244 sensitivity of Hain GQM would be valuable.

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29 245 We acknowledge the limitations in our evaluation which include the number of
30 246 specimens tested, confining the testing to nasal and groin specimens only (other licensed
31 247 specimens include throat and wounds), the taking of only one specimen per site for both
32 248 PCR and culture and the processing of this specimen by culture prior to PCR instead of
33 249 vice-versa. While an evaluation of two swabs per site, i.e. one for PCR and one for
34 250 culture might have led to fewer discrepant results, this represents considerable additional
35 251 processing time and expense for routine diagnostic laboratories. Additionally, we did not
36 252 want to alter the accredited method of culture as this was an evaluation in a routine
37 253 diagnostic setting, thus minimizing any deviations from normal practice was important.
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47 254 Furthermore, discrepancies may still have occurred if two specimens had been taken, e.g.

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2 255 due to a greater load of MRSA being swabbed with the first specimen compared with the
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4 256 second. However, for evaluations of new diagnostic methods the additional expense and
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6 257 time should be incurred.
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8
9 258 In summary, while agreement of results occurred for 92.3% of specimens
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11 259 processed with culture first followed by PCR, the number of discrepant results, and
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13 260 questions posed by these results suggest that culture followed by PCR using the one swab
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15 261 is not suitable for routine use. It is unclear if an alternative swab processing regime, i.e.
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17 262 PCR followed by culture would overcome these shortcomings but this may result in a
18
19 263 reduced yield from culture. Consequently it is likely that where both culture and PCR are
20
21 264 being used, two swabs need to be taken with the resulting time and expense incurred.
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23 265

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40
41 274 from this study for any of the three study authors.
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45 46 47 277 **References**

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Culture	Positive	Negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Positive	51	38	57	100	100	92
Negative	0	451				

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Table 1: Sensitivity, specificity, PPV and NPV of the Hain GQM assay.

For Peer Review