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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:

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 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 (1.7x104CFU/ml) when MRSA suspensions were used for DNA extraction or 1.4x103 CFU/swab (1.4x104 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.

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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Keywords: MRSA, Rapid Detection, PCR, Screening specimens.

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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 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 ($1.7x10^4$CFU/ml) when MRSA suspensions were used for DNA extraction or $1.4x10^3$ CFU/swab ($1.4x10^4$ 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.

Introduction

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

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

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

Methods

Bacterial isolates

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

Conventional culture and MRSA identification

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

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

Limit of detection (LoD) assays

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

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

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

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

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GQM assay inhibition by CA agar

To establish if culturing screening specimens on CA prior to GQM processing resulted in any PCR reaction inhibition the following experiment was carried out (n=3). A 10-fold dilution series of a 0.5 M^{c}Farland *S. aureus* ATCC 43300 was made in 1ml volumes of sterile saline. 40 µl of each dilution was added to 260 µl of GQM buffer Q-LYS. A duplicate set of dilutions was prepared. In the first set, a sterile Copan swab was rubbed on a CA plate just before washing in each 300 µl dilution and processing by GQM. In the second set, a sterile Copan swab that had not been exposed to CA agar was washed in each 300 µl dilution and processed by GQM. The number of CFU per dilution was determined by spread plating (as for LoD above). LoD results were compared to determine if rubbing the swab on CA prior to PCR had an inhibitory affect.

GQM version 2.0

All tests were performed according to the manufacturer's instructions except that specimens were cultured on CA prior to washing the swab in lysis buffer. The PCR amplification mix consisted of 35 µl primer nucleotide mix, 5 µl 10x polymerase buffer, 0.5 µl of 25mM $MgCl_2$ 0.3 µl Fast start Taq (5u per µl) (Roche), 5 µl DNA template and 4.2 µl water. The amplification protocol was according to the manufacturers instructions.

Patient specimen collection and processing

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

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Chromogenic agar (CA) inhibition

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

Results

Analytical sensitivity and specificity

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

LoD assays

Using a saline dilution series and plate counting, the LoD when MRSA suspensions were used directly for DNA extraction and subsequent GQM amplification was determined to be 704 CFU/GQM reaction (equivalent to a calculated $1.7x10^4$ CFU/ml). As only 5 µl of the extracted 300 µl DNA preparations can be used as template in the GQM PCR reaction, it can be said the LoD per PCR is 11.8 CFU.

The LoD when MRSA suspensions were absorbed onto Copan screening swabs prior to DNA extraction and subsequent GQM amplification was determined to be $1.4x10^3$ CFU/swab equivalent to a calculated $1.4x10^4$ CFU/ml.

Chromogenic agar inhibits the Hain® GQM assay

In a GQM reaction where a swab was washed in GQM buffer Q-LYS containing a known number of MRSA cells, the lowest number of MRSA detected was 1,390 CFU/reaction. In contrast, where the swab was first rubbed on a CA plate and then washed in GQM buffer Q-LYS containing the same number of MRSA cells as above, the lowest number of MRSA detected was 5,560 CFU/reaction, suggesting processing of swabs on CA prior to GQM PCR does have an inhibitory effect and increases LoD per reaction or CFU/ml by a factor of 4 (approximately).

Clinical specimens

Of 540 specimens processed 47/540 (8.7%) were both PCR and culture positive and 451/540 (83.5%) were negative by both methods. Discrepant results occurred with 42/540 (7.7%) specimens; 4 were PCR positive but culture negative and 38 were PCR negative but culture positive. All four patients with a positive PCR and culture negative result had been decolonised and thus were considered true MRSA positives. The amended MRSA positive rate was therefore calculated as 51/540 (9.4%), with a discrepant rate of 38/540 (7.0%). For these remaining 38 discrepant results (PCR negative/culture positive specimens), PCR rechecks of the prepared DNA extracted/lysis reactions were carried out and all were confirmed negative.

All 38 swabs were plated a second time on CA and subsequently enriched in TSB broth for 24 h followed by plating on CA. Twelve grew after direct plating and enrichment, suggesting the microorganisms were present on the swabs but were not detected by PCR. PCR on all 12 of these isolates (colony tested) were PCR positive, therefore these were considered true PCR false negatives. *S. aureus* was not isolated again after repeat culture with enrichment in TSB for the remaining 26/38 discrepant specimens. These were considered false negatives. The sensitivity, specificity, positive predicative value (PPV) and negative predictive value (NPV) were calculated (Table 1).

Discussion

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

The LoD of GQM can be reported in a number of ways. Here we established the LoD of the complete GQM assay to be 704 CFU. This is the minimum number of MRSA cells required to be added to the GQM extraction mix that will result in a positive hybridization signal. LoD can also be expressed as LoD per PCR and has this has been calculated as 11.8 CFU per PCR. As many studies report LoD of rapid assays in CFU per ml or CFU per swab these GQM LoD results can also be reported as $1.4x10^4$ CFU/ml or $1.7 x10^3$ CFU/swab. By contrast, the LoD of other rapid methods have been reported as 6.1 $x10^2$ CFU/ml or 5.8 $x10^1$ CFU/swab for GeneXpert and $1.7x10^2$ CFU/swab for routine MRSA chromogenic culture [4].

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

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

positive because inoculum levels were below the LoD of the GQM assay. Depending on the interpretation of these findings various sensitivities could be calculated. If we excluded the 26 specimens from sensitivity calculations that were GQM negative, culture positive from the analysis, assuming that because of the work flow used all organisms had been removed by culture prior to PCR or CA inoculation resulted in inhibition, a GQM sensitivity of 81% would be achieved. If we included the 26 specimens in the sensitivity calculation but assumed that all 26 specimens would have been PCR positive/culture positive if specimens had been processed by PCR first followed by culture, then an improved sensitivity of 87% would result. However, the actual sensitivity determined here was 57%. The true value for the sensitivity value is at best between 81%-87% but at worst between 57-81%, depending on the interpretation of the PCR negative culture positive discrepant results. A full study to establish the diagnostic sensitivity of Hain GQM would be valuable.

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

due to a greater load of MRSA being swabbed with the first specimen compared with the second. However, for evaluations of new diagnostic methods the additional expense and time should be incurred.

In summary, while agreement of results occurred for 92.3% of specimens processed with culture first followed by PCR, the number of discrepant results, and questions posed by these results suggest that culture followed by PCR using the one swab is not suitable for routine use. It is unclear if an alternative swab processing regime, i.e. PCR followed by culture would overcome these shortcomings but this may result in a reduced yield from culture. Consequently it is likely that where both culture and PCR are being used, two swabs need to be taken with the resulting time and expense incurred.

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

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