Evaluation of a Liquid Medium Transport Swab (Sigma-Transwab®) for the Detection of MRSA Using the Cepheid GeneXpert® PCR Analysers

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Abstract

Background

With recent advances of automation within microbiology, such as automated plate spreading systems, the use of liquid transport swabs is increasing. It would be advantageous if these swabs could be used in the molecular tests such as PCR which are now widely used for rapid turnaround of urgent samples. However these tests are often only validated for the manufacturer’s dedicated collection device. ASM’s Cumitech 31A, provides a method for validation of non-specified devices, and was used as the basis of this study to evaluate the performance of a liquid medium transport swab (Sigma Transwab® from MWE) in the Cepheid GeneXpert® PCR system for MRSA in comparison to the dedicated Cepheid collection device (Copan Duo Swab).

Methods

Inoculum

A 0.5 McFarland suspension of MRSA (wild type strain control) was prepared and diluted 10\(^{-1}\), 10\(^{-2}\), 10\(^{-3}\), and 10\(^{-4}\). For Sigma Transwab®, 100µl of suspension (for each dilution) was pipetted into the medium and vortexed. 100µl of medium was then pipetted into lysis buffer provided, vortexed again and all of the lysis buffer solution placed into the GeneXpert® cartridge, and processed. A total of 50 Sigma Transwabs® were processed.

For the Copan swabs, 100µl of suspension for each dilution was pipetted into a microtitre well. The collection swab was allowed to absorb the solution, then broken off into the lysis buffer, vortexed and all the lysis buffer solution placed into the GeneXpert®, and processed. A total of 50 Copan Duo Swabs were processed.

100 Negative samples using sterile saline were also tested for each device type.

Results

All 200 negative samples gave negative results.

In total 100 tests were performed. 50 for the Copan swab and 50 for the Sigma swab over the course of three days. The average over the three days for both swabs cycle threshold (CT) value for the Meca gene was plotted on a graph against the dilution concentration to show that both swabs are comparable in their results (Fig. 1).

Conclusion

Sigma-Transwabs® and Copan swabs detected MRSA organism at all dilutions, with similar CT values. At relatively low concentrations of organism, PCR analysis can be performed readily from Sigma Transwab®. Sigma Transwab® performed just as well as the Copan swab, but is also available for further testing with other methods and for other pathogens unlike the Copan device.

Methods

Table 1

<table>
<thead>
<tr>
<th>Collection Device</th>
<th>Sigma Transwab</th>
<th>Cepheid/Copan Duo Swab</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA Suspension</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Saline</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Sigma Transwab</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>

Methods used for inoculation

The two devices on trial are used in different ways. The Cepheid/Copan collection device sold for use with the GeneXpert® is a double swab which is used to collect the specimen (Ref: Cepheid MRSA Specimen Collection Protocols). The entire swab bud is placed into the sample reactive vial, which is vortexed and the contents dispensed into the specimen port of the GeneXpert® Test Cartridge (GeneXpert®). A minimum of 100µl of suspension must be dispensed into each device tested, 100µl of MRSA suspension, or 100µl of saline was dispensed into the well of a microplate. The entire contents of the swab were absorbed onto the bud of the swab from the collection device. The bud was then snapped into the sample reactive vial for processing. With the presence of liquid medium transport swabs, a method is that an aliquot of sample will be added to the sample reactive vial, with the remainder retained for alternative testing if required. For this study a 100µl aliquot of MRSA suspension, or 100µl of saline was added directly into the Sigma Transwab® tube. This was shaken, and a 100µl aliquot of the sample reactive vial which was then vortexed, and the contents dispensed into the specimen port of the GeneXpert® cartridge.

One of the implications of this protocol is that the Sigma Transwab® sample is effectively subjected to an additional dilution step. This could be seen as disadvantageous for the Sigma Transwab® in this comparison, it is justified as showing how the device would be used in clinical practice. The purpose of this study was to determine if the Sigma Transwab® medium (liquid Amies) causes any interference with the PCR process of the GeneXpert® system for MRSA, when compared with the Cepheid/Copan device, as it was important for both systems to have an identical challenge of MRSA being detected. This is consistent with Cepheid’s own data for the Limit of Detection for the collection of specimens for MRSA screening, and it would be useful if these could also be used with PCR systems such as GeneXpert® for confirmation, or rapid testing in acute cases. In this study the Sigma-Transwabs® were able to detect the MRSA organism at all the dilutions used. The CT values at each dilution for both swabs were similar in their readings. In fact Sigma-Transwab® has effectively caused a further dilution because of the adding of the suspension to the liquid medium, and is yet able to match the Cepheid/Copan controls. The lowest concentration, (dilution 10\(^{-4}\)) still produced a reliable result from the Sigma-Transwabs® with MRSA being detected. This is consistent with Cepheid’s own data for the Limit of Detection for this PCR method, and the CT values were comfortably within the stated range for reliability. For lower concentrations the sensitivity can easily be increased by taking a larger aliquot from the Sigma-Transwab®. In a similar type of study also reported at this meeting, Silbet at al used 500µl of liquid medium sample to ensure detection of the lowest concentrations. This study has shown that even low concentrations of organism, PCR analyses can be performed directly from the liquid transport medium of the Sigma-Transwab®. The Sigma-Transwab® also performed just as well as the Cepheid/Copan collection kit, with the added advantage of being able to use the same swab on automated platforms for conventional diagnostic testing.

References

1. Cumitech 31A, Verification & Validation Procedures in the Clinical Microbiology Laboratory (ASM, 2009)