EVALUATION OF SIGMA TRANSWAB® (LIQUID MEDIUM SWABS) AND CEPHEDI/ CEPHIDI DUO SWAB FOR THE RAPID DETECTION OF MRSA USING THE GENEXPERT® PCR ANALYSER

Kamran Khan & Helen Jones
NHS Heathwood & Wexham Park Hospital, Slough, United Kingdom

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 specimens. However, these tests are often only validated for the manufacturer’s dedicated collection device. ASMs 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 device (Sigma Transwab® from MIWE) in the Cepheid GeneXpert® PCR system for MRSA in comparison to the dedicated Cepheid collection device (Cepheid/Copan Swab). Method

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®, and processed. A total of 50 Sigma Transwabs® were prepared.

For the Copan swabs, 100µl of suspension for each dilution was pipetted into a microtome tray 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 organisms 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.

Introduction

With recent advances of automation within microbiology, such as automated plate spreading techniques, the use of liquid swabs has become popular. Liquid medium transport swabs are known to work well for the culture of specimens onto solid media; however PCR analysis can be performed readily from Sigma Transwab®. Randomised swabs have also been demonstrated in spreading techniques, the use of liquid swabs has become popular. Liquid medium transport swabs are known to work well for the culture of specimens onto solid media; however PCR analysis is also becoming routine, especially for urgent samples and with added convenience of not going through solid media. 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 specimens.

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 Protocol®). The entire swab is placed into the lysis buffer provided, vortexed again and all of the lysis buffer solution placed into the GeneXpert®, and processed. For the study, for each device tested, 100µl of Sigma Transwab® suspension, or 100µl of saline was dispensed into the well of a microtome tray. The entire contents of the well were absorbed onto the bud of one of the swabs from the collection device. The bud was then snapped into the lysis buffer for PCR processing.

With the Sigma Transwab®, the intended method is that an aliquot of sample will be added to the sample reagent vial, which is vortexed and the contents dispensed into the specimen port of the GeneXpert® Test Cartridge (Xpert MRSA/SA Nasal G3). For the study, for each device tested, 100µl of Sigma Transwab® suspension, or 100µl of saline was dispensed into the well of a microtome tray. The entire contents of the well were absorbed onto the bud of one of the swabs from the collection device. The bud was then snapped into the lysis buffer for PCR processing.

Results

Negative controls

100 negative samples consisting of 100µl saline were run for each device. All tests correctly reported a negative result. There were no false positives.

Positive

50 positive samples consisting of 100µl suspension of a known MRSA strain were run for each device. Each sample was correctly identified as positive for the MecA gene, the identifier used by the GeneXpert® MRSA test system. There were no false negatives with either test device.

For both systems, the average 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).

At the 10^-1 dilution the average CT value was 23.4 for the Sigma Transwab® and 24.4 for the Copan device.

At the 10^-4 concentration the average CT value was 32.3 for the Copan device and 32.5 for the Sigma Transwab®.

Conclusion

The study shows that even at relatively low concentrations of organism, PCR analysis can be performed directly from the liquid transport medium of the Sigma-Transwab®. In clinical practice, both systems may collect larger specimens than the Cepheid/Copan collection device, so it was important for both systems to have an identical challenge of 100 µl of MRSA suspension. The lowest concentration, (dilution 10^-4), still produced a reliable result from the Sigma-Transwab®. The Sigma-Transwabs® 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.

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 Result</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Saline 0 100 0 100</td>
<td>50 0</td>
<td>50 0</td>
</tr>
<tr>
<td>MRSA Suspension 50</td>
<td>0 100</td>
<td>0 100</td>
</tr>
</tbody>
</table>

Fig 1

Average CT-values for positive MRSA samples

References

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