



Evaluation of Sigma Transwab® (liquid medium swabs) for the Rapid Detection of MRSA using the GeneXpert® PCR Analyser

Kamran Khan & Helen Jones

Abstract

Objectives

Recent years have seen rapid developments of technology available in the clinical microbiology laboratory. Molecular and serological techniques such as PCR, ELISA and MALDITOF allow rapid identification of specific pathogens. Automated planting is transforming conventional culture based microbiology. It is often useful to combine methods but many test platforms are validated for only one device.

Sigma Transwab® (MWE) is a liquid Amies transport swab suitable for many specimen types. Designed for use on all current automated plate and broth planting platforms, it can also be used in conventional methods, and is intended to be compatible with molecular testing. GeneXpert® PCR Analyser (Cepheid) is a molecular platform suited for the rapid identification of many important pathogens, including MRSA from nasal swabs. The GeneXpert® system is currently only validated for use with a dedicated swab device (Copan), which has to be used completely, and so unavailable for further tests.

This study was designed to show if Sigma Transwab® could be used with the GeneXpert® platform. The same specimen could be used for further testing.

Method

Testing was according to Cumitech 31A, Verification & Validation Procedures in the Clinical Microbiology Laboratory (ASM, 2009). Sigma Transwab® tubes were inoculated with dilutions (100 µl of 10⁻¹, 10⁻², 10⁻³, 10⁻⁴ dilutions of 0.5 McFarland) of MRSA control strain. After vortexing, 100 µl of medium was added to lysis buffer, vortexed again and transferred to the GeneXpert® cartridge and processed. Copan Duo swabs were also inoculated with 100 µl of the same dilutions, and processed by breaking the complete swab head into the lysis buffer, which is then vortexed and transferred to the cartridge. Negative samples were also run on each platform (as required by Cumitech).

Results

50 positive and 100 negative samples were tested for each device. For each device all negative samples were correctly identified as negative. All positive samples were also correctly identified, including genotype, with virtually identical CT values for each device. For the 10⁻⁴ dilution, the Copan device had a CT value of 32.3 and the Sigma Transwab® 32.5 (although the Sigma specimen is effectively diluted once more), Fig. 1.

Conclusion

Both devices showed comparable sensitivity for the detection of MRSA, but the Sigma Transwab® is preferred because it can be used for further pre- or post-testing for other pathogens.

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 is also becoming routine, especially for urgent samples and with the need for quicker turnaround times. Sigma liquid transport swabs (Sigma-Transwab®) from Medical Wire in comparison with the Cepheid dual swab collection kits (manufactured by Copan) were used in this study to determine if the routine liquid medium swabs can be used on the Cepheid GeneXpert platform for PCR analysis instead of the dedicated collection devices.

Method

Testing was according to Cumitech 31A, Verification & Validation Procedures in the Clinical Microbiology Laboratory (ASM, 2009).

Sigma-Transwab® A 0.5 McFarland standard solution of MRSA (wild type strain control) was used to perform serial dilutions of 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴. 100µl of the 10⁻¹ dilution was pipetted into the liquid medium of the Sigma-Transwab®, which was then vortexed. 100µl of medium was then pipetted into the lysis buffer provided for GeneXpert. After vortexing again, all of the lysis buffer solution was placed into the GeneXpert cartridge, and run on the analyser.



Cepheid (Copan) collection kit. The same dilutions were used for the Cepheid/Copan kits, with the procedure adjusted as follows.

100µl of suspension was pipetted into a well of a microtitre plate. One swab from the dual swab provided was placed in the well, and allowed to absorb all of the suspension. The swab was then broken into the lysis buffer, vortexed, and then all the lysis buffer solution was placed into a cartridge and run on the GeneXpert analyser.



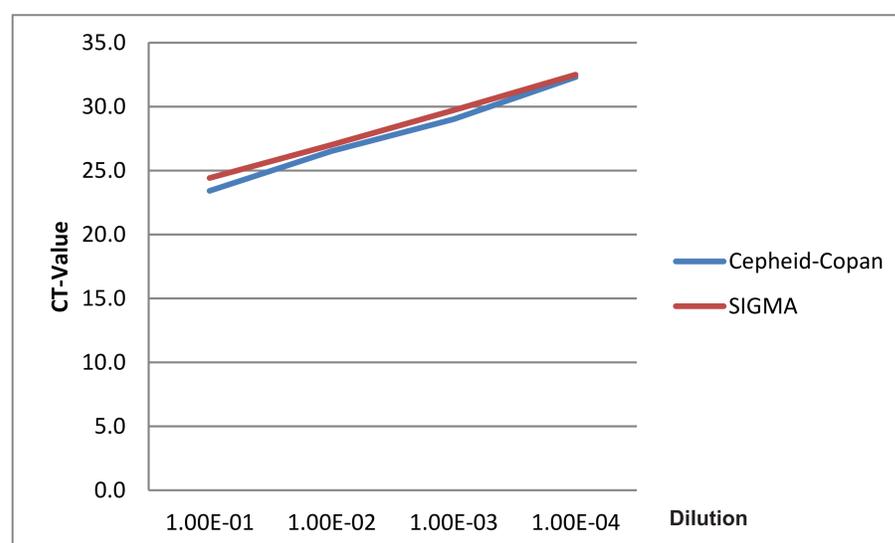
For each system, 50 known positive tests were performed as detailed, with at least 12 tests for all 4 dilutions. 100 negative samples for each swab type were also run using sterile saline and the same procedures.

Results

Of the 200 negative samples run, all were negative.

For the positive samples, in total 100 assays were performed - 50 for the Cepheid/Copan collection kits, and 50 for the Sigma Transwab®. 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. (Fig1)

At the 10⁻¹ concentration the average CT value was 23.4 for the Cepheid/Copan swab, and 24.4 for the Sigma-Transwab®. At the 10⁻⁴ concentration the average CT values were 32.3 (Cepheid/Copan) and 32.5 for Sigma-Transwab®.



Conclusion

Cumitech 31A, Verification & Validation Procedures in the Clinical Microbiology Laboratory (ASM, 2009) is a recognised method for validation of alternative (off-label) devices for use with particular analytical systems. At the time of performing the study, only the Cepheid (Copan) collection device was validated for use with GenXpert system for MRSA. However many other devices are routinely used in healthcare establishments for the collection of specimens for MRSA screening, and it would be useful if these could also be used with PCR systems such as GenXpert 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 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, 10⁻⁴, still produced a reliable result from the Sigma-Transwabs® with MRSA being detected. This study has shown that even at relatively low concentrations of organism, PCR analysis 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 such as those for inoculation and suspension.

This study was supported by MWE

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