

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

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Methods

POSITIVE

RESULTS

See Fig 1

50 Samples

100ul

0.5 McFarland standard solution of

MRSA (1.5×10^8)

Serial Dilutions:10⁻¹, 10⁻², 10⁻³, 10⁻⁴

50 Samples

100μΙ

Tech. Initial/Date Supervisor Initial/Date
* indicates that a particular field is entered using a barcode scanner

Microtitre plate



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. 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 device (Sigma Transwab® from MWE) in the Cepheid GeneXpert® PCR system for MRSA in comparison to the dedicated Cepheid collection device (Copan Duo 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 processed.

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

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-Transwab® with liquid Amies medium (Medical Wire) is such a device. It is widely used for the collection of specimens for MRSA screening using conventional methods, both manual and automated. This study was set up to compare the results obtained for Sigma Transwab® with those of the Cepheid dual swab collection kits (manufactured by Copan) on the Cepheid GeneXpert® platform for PCR analysis, and to validate the use of Sigma Transwab® on this system.



Confirmed MRSA Findings on GeneXpert

TEST METHOD: Cumitech

Procedures in the Clinical

31A. Verification & Validation

Microbiology Laboratory (ASM,

NEGATIVE

CONTROLS

100 Samples

100μl of sterile saline

Sigma Transwab®

(1ml liquid Amies medium)

_ 100μΙ

Sample Reagent

vial with lysis

Table 1

using MRSA Nasal

Collection Device	Sigma Transwab®		Cepheid/Copan Duo Swab	
MRSA Result	Positive	Negative	Positive	Negative
Saline	0	100	0	100
MRSA Suspension	50	0	50	0

Fig 1 **Average CT-values for positive MRSA samples**

in less than 1 hour.

GeneXpert® System is a modular,

cartridge based real-time PCR

concentrate, detect and identify

targeted nucleic acid sequences

specimens. The cartridges used in

this study are intended to detect

targets such as *mecA* for MRSA

(polymerase chain reaction)

system which can purify,

directly from compatible

NEGATIVE

CONTROLS

100 Samples

100μl of sterile saline

Cepheid/Copan

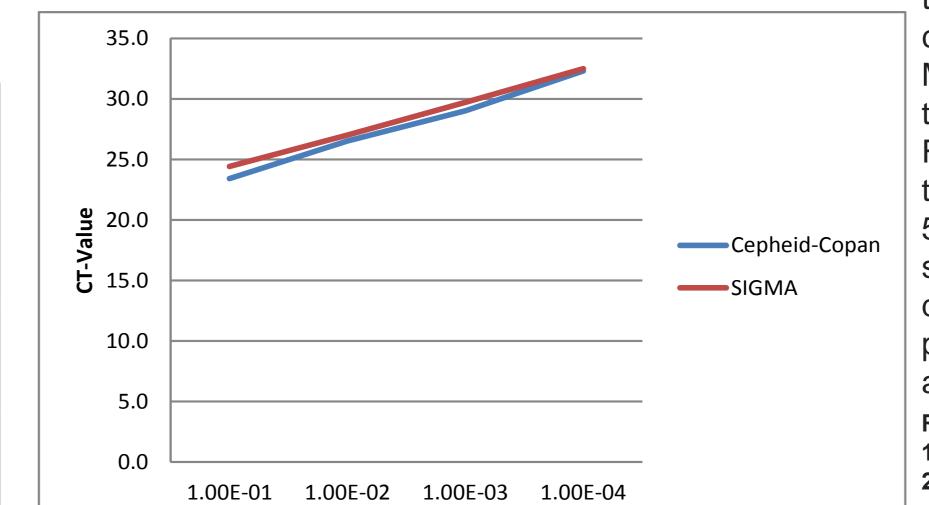
collection device (Duo

Swab). Suspension

completely absorbed

head placed in lysis

onto swab. Entire swab



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 bud is placed into 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 MRSA suspension, or 100µl of saline was dispensed into the well of a microtitre plate. 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 sample reagent vial for processing. With the Sigma Transwab[®], the intended method is that an aliquot of sample will be added to the sample reagent 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 the Sigma Transwab® tube. This was shaken, and a 100µl aliquot removed and added to the sample reagent 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 reflecting 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, so it was important for both systems to have an identical challenge of 100µl suspension or saline either completely absorbed onto the swab bud (Cepheid/Copan) which was tested in its entirety, or added to the tube of the Sigma Transwab®. In clinical practice, both systems may collect larger specimens from the two swabs in the Cepheid/Copan device, or in the double or triple swabs often supplied for MRSA testting with Sigma Transwab®.

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 mec A 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. (Fig1)

At the 10⁻¹ dilution 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[®]. All readings are within the published acceptable ranges for the GeneXpert MRSA test

Conclusion

Cumitech 31A, 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 GeneXpert ® 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 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 had 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, Silbert et al used 500µl of liquid medium sample to ensure detection of the lowest concentrations. 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 for conventional diagnostic testing.

- 1. Cumitech 31A, Verification & Validation Procedures in the Clinical Microbiology Laboratory (ASM, 2009)
- 2. Silbert et al, Poster 1801, Abstracts for ASM2013, in "The official Mobile Event App of asm2013", TriStar Publishing, KS 66213

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