VALIDATION ACCORDING TO CUMITECH 31A OF THE USE OF SIGMA TRANSWAB® AND SIGMA TRANSWAB® PF WITH THE WALK-AWAY SPECIMEN PROCESSOR WASP® (COGNIS)

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INTRODUCTION

The practice of clinical microbiology has changed rapidly in recent years, leading to the automation of plating methods and the use of suitable transport swabs. The Copan Walk Away Specimen Processor (WASP®) is an instrument for automated plating of microbiological specimens. For many laboratories receiving specimens from numerous locations, it is important that the platform can handle alternative devices which may have been manufactured by another company.

The purpose of this study was to determine that using the alternative devices Sigma Transwab® and Sigma Transwab® PF would not interfere with the working of the Copan WASP® platform, and would allow the correct identification of pathogens, with identical results to those obtained using Copan’s own e-swab® devices.

The study followed the guidance set out in Cumitech 31A, Verification and Validation of Procedures in the Clinical Laboratory (American Society for Microbiology), which describes laboratories’ own performance verification specifications for new tests or consumables prior to the reporting of patient test results. These performance specifications are defined as accuracy, precision, and reportable range all compared with results obtained for a reference range, typically the test system manufacturer’s own defined values.

The study was designed to meet the requirements of Cumitech 31A, (ASM). The purpose was to validate the use of Sigma Transwab® and Sigma Transwab® PF for the processing of microbiological specimens on the Copan WASP®. The basic requirement is to show matching results for a minimum of 50 known positive samples and 100 known negative samples tested with the trial device (in this case Sigma Transwab® and Sigma Transwab® PF) when compared with the results obtained for the WASP® manufacturer’s own validated product (e-swab®).

In addition to the validation of the new devices, the study was also used to further assessment of performance in terms of accuracy, precision and reportable range.

In conclusion the new test method was successfully performed the method is parallel with an established and reliable reference method. In this study it has been shown that using any device on the WASP® allow microbiological specimens to be processed with similar results to those obtained using some of the swabs manually to confirm that the organism is indeed present, and it also it correctly retrieved from plates processed on the WASP®.

The precision of a method is a measure of its ability to produce exactly the same result when the test is repeated at different times. In the present study this is done by comparing results obtained for identical samples at different stages of a run on the machine, and also by repeating the test using identical samples on a different run.

An indicator of the reportable range for the test is given by running samples inoculated at the lower and higher concentrations of the microorganisms. All results are compared with those obtained for the reference range, which in this case were the Copan e-swab® results.

METHODS

Standard inoculation of overnight culture of ATCC control strains (m/v) were prepared in accordance with ISO 6888-4 and tested in the final amount of inoculating bacteria of approximately 10⁵ and 10⁶ CFU by swab. Results were processed as follow:
- For E. coli 0.5 McFarland suspension was diluted 1:10 (Solution 1EC) followed by further 1:10 dilutions (2EC and 3EC). 10 wells were prepared with 2EC, 36 wells with 3EC and 10 wells with 3EC.
- For Staphylococcus aureus 0.5 McFarland suspension was diluted 1:10 followed by further 1:10 dilutions (Solution 1SA). 20 wells were prepared with 1SA.
- For Staphylococcus aureus 0.5 McFarland suspension was diluted 1:10 followed by further 1:10 dilutions (Solution 2SA). 20 wells were prepared with 2SA.
- For Staphylococcus aureus 0.5 McFarland suspension was diluted 1:10 followed by further 1:10 dilutions (Solution 3SA). 20 wells were prepared with 3SA.
- For negative controls a further 100 wells were each prepared with 15µl sterile water.

Inoculation of wells

For each concentration the inoculated plates were removed from the pack, allowed to absorb completely all the inoculum in the microtitre plate wells, then placed into its respective transport tube, the shaft snapped at the breakpoint and the tube piped in accordance with the manufacturer’s instructions. In all cases the inoculum used was 15µl suspension in the well of a microtitre plate. The swabs were inoculated as shown in Table 2.

Following inoculation, 12 of each of the 2EC inoculated plates and 22 of each of the wells with sterile water were placed in the fridge (4°C) to be retained until producing the following day in Run 2.

RESULTS

Swabs processed on WASP

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Sigma Transwab®</th>
<th>Sigma Transwab® PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli 0.5 McFarland</td>
<td>100% 100%</td>
<td>100% 100%</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>100% 100%</td>
<td>100% 100%</td>
</tr>
<tr>
<td>Negative control</td>
<td>100% 100%</td>
<td>100% 100%</td>
</tr>
</tbody>
</table>

Processing of wells

The swabs were processed on the WASP® using manufacturer's instructions, in accordance with the following order. The WASP® instrument was also loaded with the appropriate age plates for each microorganism.

For each dilution and sterile water, a further 2 wells of each kind were processed manually.

<table>
<thead>
<tr>
<th>Run 1</th>
<th>Sigma Transwab®</th>
<th>Sigma Transwab® PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli 0.5 McF</td>
<td>50% 50%</td>
<td>50% 50%</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>50% 50%</td>
<td>50% 50%</td>
</tr>
<tr>
<td>Negative</td>
<td>50% 50%</td>
<td>50% 50%</td>
</tr>
</tbody>
</table>

Table 1. Bacteria used in the study

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>ATCC Control Strain</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>ATCC 25922</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>ATCC 19660</td>
<td></td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>ATCC 10899</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC 27853</td>
<td></td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>ATCC 14028</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>ATCC 19433</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>ATCC 19115</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

This was a large-scale study involving the processing of 600 separate sample wells, 50% of these on the Copan WASP®, and 60 tested manually as controls for the procedure. One of the aims of the study was in addition to the validation of the use of different kinds of devices, was to demonstrate that the WASP® would run correctly and without interruption using the different kinds of device. All the devices used were intended to be compatible with the automatic operation of the WASP®. In this case all devices ran correctly with no mechanical problems arising.

The study was designed to meet the requirements of Cumitech 31A, (ASM). The purpose was to validate the use of Sigma Transwab® and Sigma Transwab® PF for the processing of microbiological specimens on the Copan WASP®. The basic requirement is to show matching results for a minimum of 50 known positive samples and 100 known negative samples tested with the trial device (in this case Sigma Transwab® and Sigma Transwab® PF) when compared with the results obtained for the WASP® manufacturer’s own validated product (e-swab®).

In addition to the validation of the new devices, the study was also used to further assessment of performance in terms of accuracy, precision and reportable range.

Accuracy is determined by the ability of the test to correctly identify the microorganisms. In the present study different species of bacteria were used. The reference point for these was the results of testing the samples on Copan e-swab® using a conventional plating method. For both E. coli 0.5 McFarland 30 devices of each type were inoculated for processing on the WASP®, with a further 4 of each type inoculated at the same time for manual processing, for results of the latter 4 the manual results, 50 devices of each type were inoculated for processing on the WASP®, and a further two for manual processing. In addition 100 devices of each type were inoculated with sterile water, and a further 4 of each type for manual processing.

When processed on the WASP®, the correct bacteria were recovered from each device, satisfying exactly the bacteria recovered from the device processed manually. There was complete agreement for all devices. Similarly nothing was recovered from any of the negative controls.

Thus all the devices tested gave completely accurate results for the test samples and controls, and can be used to comply with the requirements of Cumitech 31A.

Precision is determined by the ability of the system to repeat the test and consistently deliver similar results. In the present study the devices inoculated with the middle concentration of E. coli were tested at the beginning and end of the first run, and again on the second run the following day. E. coli was correctly recovered from devices at both stages of the first run, and on the second run. Nothing was recovered from the corresponding negative controls for either run. The test devices (Sigma Transwab® and Sigma Transwab® PF) could be used to operate on the WASP® with equivalent precision to that of the previously validated e-swab®.

The reportable range for this study was determined for E. coli samples using three dilutions of organism. In the present study the devices inoculated with the middle concentration of E. coli were tested at the beginning and end of the first run, and again on the second run the following day. E. coli was correctly recovered from devices at both stages of the first run, and on the second run. Nothing was recovered from the corresponding negative controls for either run. The test devices (Sigma Transwab® and Sigma Transwab® PF) could be used to operate on the WASP® with equivalent precision to that of the previously validated e-swab®.

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A total of 570 swabs were run and correctly processed on this platform. Sigma Transwab® and Sigma Transwab® PF have been designed to be run on any of the currently available automated platforms, and this study has validated their use with the WASP®.

Acknowledgements

We wish to thank all Biotechnical members of the Microbiology Department for their technical support during the study. This study and presentation was supported by Medical Wire.

References


For media wire, visit: www.laboratoires-biogroup.com - n.chatelain@labo-biogroup.com