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

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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 target pathogens, with identical results to those obtained using Copan's own e-swab® devices.
The study follows the guidance set out in Cumitech 31A, Verification and Validation of Procedures in the Clinical Laboratory (American Society for Microbiology), which describes how laboratories can verify performance 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 branded devices.
The accuracy of a test method is assessed by performing the method in parallel with an established and reliable reference method. In this study it has to be shown that using any device on the WASP® allows any microorganism contained in the specimen to be correctly identified. This is done by plating some of the swabs manually to confirm that the organism is indeed present, and that it is also correctly recovered 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 indication 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®.

MATERIALS

The swab devices used for this study were :
Medical Wire Sigma Transwab® with Liquid Amies (with standard foam-tip swab)
Product Code MW176S
Medical Wire Sigma Transwab®PF with liquid Amies (with standard PurFlock® tip swab)
Product Code MW176PF
Copan e-swab® with Liquid Amies with flocked swab
Product Code 480CE

The bacteria (as shown in Table 1) were chosen to represent some typical specimens the laboratory would routinely have to process, and allow an effective validation of the use of the test devices on this platform.

Table 1. Bacteria used in the study

ATCC Control strains	Abbreviation for this study
Escherichia coli ATCC 25922	EC
Staphylococcus aureus ATCC 29213	SA
Klebsiella pneumoniae ATCC 700603	KP
Pseudomonas aeruginosa ATCC 27853	PA
Enterococcus faecalis ATCC 29212	EF


Walk-away Specimen Processor (WASP ®)


Sigma Transwab


Sigma Transwab PF


e-swab

METHODS

Standard inoculum of overnight culture of ATCC control strains (n=5) were prepared in accordance with CLSI M40-A2 standard resulting in the final amount of inoculating bacteria of approximately 10^5 and 10^6 CFUs by swab. Inocula were prepared as follows :
- For EC a 0.5 McFarland suspension was diluted 1:5 (Dilution 1EC) followed by 2 further 1:10 dilutions (2EC and 3EC). 30 wells were prepared with 1EC, 90 wells 2EC, and 30 wells with 3EC
- For SA a 0.5 McFarland suspension was diluted 1:5 followed by 1 further 1:10 dilution (Dilution 2SA). 30 wells were prepared with 2SA.
- For KP a 0.5 McFarland suspension was diluted 1:5 followed by 1 further 1:10 dilution (Dilution 2KP). 30 wells were prepared with 2KP.
- For PA a 0.5 McFarland suspension was diluted 1:5 followed by 1 further 1:10 dilution (Dilution 2PA). 30 wells were prepared with 2PA.
- For EF a 0.5 McFarland suspension was diluted 1:5 followed by 1 further 1:10 dilution (Dilution 2EF). 30 wells were prepared with 2EF.
- For negative controls a further 300 wells were each prepared with 50µl sterile water.

Inoculation of swabs
For each test point the swab was removed from its pack, allowed to absorb completely all of the inoculum in the microtitre plate well, then placed into its respective transport tube, the shaft snapped at the breakpoint and the tube capped in accordance with the manufacturer's instructions. In all cases the inoculum used was 50µl of suspension in the well of a microtitre plate. The swabs were inoculated as shown in Table 2.
Following inoculation, 12 each of the 2EC inoculated swabs and 22 each of the swabs with sterile water were placed in the fridge (2 – 8°C) to be retained until processing the following day in Run 2.

Table 2. Numbers of swabs inoculated

Dilution	Sigma Transwab®	Sigma Transwab® PF	e-swab®
1EC	12	12	12
2EC	34	34	34
3EC	12	12	12
2SA	12	12	12
2KP	12	12	12
2PA	12	12	12
2EF	12	12	12
Sterile water	104	104	104

Processing of swabs
The swabs were processed on the WASP®, as per manufacturer instructions, in two runs in the following order. The WASP® instrument was also loaded with the appropriate agar plates for each microorganisms.
For each dilution and for sterile water, a further 2 swabs of each kind were processed manually.
Total recovery of viable bacteria was expressed with a decimal logarithm of recovered CFUs and compared to those processed manually.

Run 1

Load	Dilution	Sigma Transwab®	Sigma Transwab® PF	e-swab®
1	1EC	5	5	5
2	2EC	10	10	10
3	3EC	5	5	5
4	2SA	5	5	5
5	2KP	5	5	5
6	2PA	5	5	5
7	2EF	5	5	5
8	Sterile Water	40	40	40
9	1EC	5	5	5
10	3EC	5	5	5
11	2SA	5	5	5
12	2KP	5	5	5
13	2PA	5	5	5
14	2EF	5	5	5
15	2EC	10	10	10
16	Sterile Water	40	40	40

Run 2

17	2EC	10	10	10
18	Sterile Water	20	20	20

RESULTS

Swabs processed on WASP

	Sigma Transwab®				Sigma Transwab® Pf				e-swab®			
Dilution	No Tested	Positive	Negative	False Results	No Tested	Positive	Negative	False Results	No Tested	Positive	Negative	False Results
1EC	10	10	0	0	10	10	0	0	10	10	0	0
2EC	30	30	0	0	30	30	0	0	30	30	0	0
3EC	10	10	0	0	10	10	0	0	10	10	0	0
2SA	10	10	0	0	10	10	0	0	10	10	0	0
2KP	10	10	0	0	10	10	0	0	10	10	0	0
2PA	10	10	0	0	10	10	0	0	10	10	0	0
2EF	10	10	0	0	10	10	0	0	10	10	0	0
Water	100	0	100	0	0	0	100	0	0	0	100	0

Swabs processed manually

	Sigma Transwab®				Sigma Transwab® Pf				e-swab®			
Dilution	No Tested	Positive	Negative	False Results	No Tested	Positive	Negative	False Results	No Tested	Positive	Negative	False Results
1EC	2	2	0	0	2	2	0	0	2	2	0	0
2EC	2	2	0	0	2	2	0	0	2	2	0	0
3EC	2	2	0	0	2	2	0	0	2	2	0	0
2SA	2	2	0	0	2	2	0	0	2	2	0	0
2KP	2	2	0	0	2	2	0	0	2	2	0	0
2PA	2	2	0	0	2	2	0	0	2	2	0	0
2EF	2	2	0	0	2	2	0	0	2	2	0	0
Water	2	0	2	0	0	0	2	0	0	0	2	0

Precision
Swabs processed at different stages

	Sigma Transwab ®				Sigma Transwab ® Pf				e-swab ®			
Dilution	No tested	Positive	Negative	False Results	No tested	Positive	Negative	False Results	No tested	Positive	Negative	False Results
2EC												
Run 1 beginning	10	10	0	0	10	10	0	0	10	10	0	0
2EC												
Run 1 end	10	10	0	0	10	10	0	0	10	10	0	0
2EC												
Run 2	10	10	0	0	10	10	0	0	10	10	0	0

Reporting range
1EC = high concentration 2EC = medium concentration 3EC = low concentration

	Sigma Transwab®				Sigma Transwab® Pf				e-swab®			
Dilution	No Tested	Positive	Negative	False Results	No Tested	Positive	Negative	False Results	No Tested	Positive	Negative	False Results
1EC	10	10	0	0	10	10	0	0	10	10	0	0
2EC	30	30	0	0	30	30	0	0	30	30	0	0
3EC	10	10	0	0	10	10	0	0	10	10	0	0

Reference range
Results for Sigma Transwab® & Sigma Transwab® Pf compared with results for e-swab®

	Sigma Transwab®			Sigma Transwab® Pf			e-swab®		
Dilution	No Tested	Positive	Negative	No Tested	Positive	Negative	No Tested	Positive	Negative
1EC	10	10	0	10	10	0	10	10	0
2EC	30	30	0	30	30	0	30	30	0
3EC	10	10	0	10	10	0	10	10	0
2SA	10	10	0	10	10	0	10	10	0
2KP	10	10	0	10	10	0	10	10	0
2PA	10	10	0	10	10	0	10	10	0
2EF	10	10	0	10	10	0	10	10	0
Water	100	0	100	0	0	100	0	0	100

DISCUSSION

This was a large study involving the processing of 630 separate swab samples, 570 of these on the Copan WASP®, and 60 tested manually as controls for the procedure. One of the aims of the study, in addition to the validation of the use of the different kinds of devices, was to demonstrate that the WASP® would run smoothly and without interruption using the different kinds of device. All the devices used are intended to be compatible with the automatic capping and de-capping mechanism on the WASP®. In this case all devices did run smoothly 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® or Sigma Transwab®PF) when compared with the results obtained for the WASP manufacturer's own validated product (e-swab®).
In fact for this study 90 known positive samples were used, to allow further assessment of performance in terms of accuracy, precision and reportable range.

Accuracy is determined by the ability of the test system to correctly identify the microorganisms. In the present study 5 different species of bacteria were used. The reference point for these was the results of testing the same bacterial suspensions using a manual plating method. For E.coli ATCC 25922, 50 devices of each type were inoculated for processing on the WASP®, with a further 6 of each type inoculated at the same time for manual processing. For each of the other 4 bacterial species, 10 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, matching exactly the bacteria recovered from the devices 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 said 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 all devices at both stages of the first run, and on the second run. Nothing was recovered from the corresponding negative controls for either run. Thus the test devices (Sigma Transwab® and Sigma Transwab®PF) could be said 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 for samples using three dilutions of organism. E.coli was correctly recovered from all samples on all devices.

Finally all positive and negative results were compared for the test devices ((Sigma Transwab® and Sigma Transwab®PF) with those obtained for the reference products which were the Copan e-swabs®. There was complete correspondence for all samples containing bacteria, and for all the negative controls.

The present study was designed to establish that these alternative devices, Sigma Transwab® and Sigma Transwab® PF can be used with confidence on the Copan WASP® platform for the processing of microbiological specimens. 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®.

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References

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