P057

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Evaluation of a new collection and enrichment swab device for direct testing in accordance with Dutch guideline on the laboratory detection of methicillin-resistant *Staphylococcus aureus*

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Introduction

One of the routine procedures in the diagnosis of infections involves the collection and transportation of a clinical swab specimen from the patient to the laboratory. In particular, for swab specimens which are to be tested for methicillin resistant *Staphylococcus aureus* (MRSA), the first stage of processing is often to transfer the swab to an enrichment broth containing sodium chloride (NaCl). Most strains of *Staphylococcus aureus* (*S. aureus*) will grow in the presence of NaCl, while other organisms commonly picked up on swabs will be inhibited. In the Dutch guidelines for laboratory detection of MRSA, a NaCl concentration of 6.5% (w/v) in an otherwise non-inhibitory broth such as tryptic soy broth (TSB) is recommended. In MWE's Sigma-TSB™ with 6.5% NaCl, those two stages are combined into one device, so that for specimens intended only to be tested for MRSA, the swab specimen is collected directly into a transport tube containing TSB with 6.5% NaCl. Following incubation, the broth is inoculated directly on to a suitable chromogenic agar, and any MRSA or methicillin sensitive *Staphylococcus aureus (*MSSA) colonies are readily identified.

The present study was designed to evaluate the ability of this device to recover and enrich *S. aureus*, while inhibiting *Escherichia coli* (*E. coli*). In many laboratories confirmation of presumptive positive MRSA is often performed using PCR, so the suitability of the swab device for such testing was also evaluated using the BD Max™System real-time PCR technology based platform.

Methods

The performance of the new device was evaluated for two swab types. Sigma® swabs with polyurethane foam buds are supplied with the standard version of the product, but an alternative version with a PurFlock® flocked polyester fibre bud is also available, and was included in the study.

Recovery and enrichment

- Suspensions of 8 *Staphylococcus aureus* and 1 *Escherichia coli* were prepared to a concentration of 10^6 CFU/ml. Serial dilutions, 10^{-1}, 10^{-2}, 10^{-3}, were made. 50µl (Sigma®) or 100µl (PurFlock®) aliquots were dispensed into the wells of a microtitre plate for each swab to be tested, for each organism / dilution / holding combination. Inoculum amount in accordance with CLSI M40-A2 Approved Standard.
- 100µl of each suspension was inoculated onto a Tryptic Soy Agar (TSA) control plate and incubated at 37° C for 24 hours.
- Three swabs were inoculated and placed in their tubes for time 0, and for each holding condition (37°C and Room Temperature for 24 hours).
- After the holding times (time 0 and 24hr) The tubes for the time 0 swabs were vortexed for 15 seconds, then using a pipette 100µl of the liquid medium was inoculated onto an a fresh TSA plate. The plates were incubated at 37°C for 24 hours.
- The results for all plates were recorded and used to calculate the effective concentration of organism for each swab type/time/ holding condition combination.

Molecular

Sigma-TSB ™

- A 0.5 McFarland suspension of a previously isolated MRSA strains was prepared and serially diluted. 50µl aliquots of 10^{-2} dilution were dispensed into the wells of a microtitre plate.
- 11 Sigma-TSB™ with Sigma® Swab and 11 Sigma-TSB™ with PurFlock Swab were inoculated by immersing the swab into the wells of a microtitre plate and absorbing as much as possible of the suspension.
- 6 of each type of inoculated swabs (Sigma® and PurFlock®) were placed in fridge and held at 4°C pending testing. These were the time zero swabs.
- 5 of each type of inoculated swabs (Sigma® and PurFlock®) were incubated at 37°C for 24 hours.
- In addition 1 uninoculated tube from each swab type was incubated at 37°C for 24 hours as a negative control.
- After the holding period all the tubes were tested by vortexing, and taking 100µl of the medium by pipette and placing into a PCR tube for processing on BD Max™. All 24 tubes, including the two negative controls, were tested with the BD Max™ MRSA XT panel.

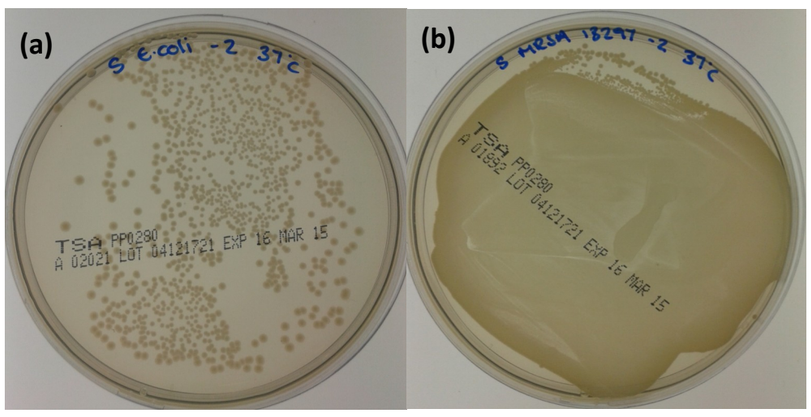
Results

Recovery and Enrichment

Table 1. Recovery and Enrichment of *Staphylococcus aureus* strains including MRSA with Sigma-TSB™ with 6.5% NaCl. RT - Room Temp, TNTC - Too numerous to count. Results shown as CFU/ml

Strain	Sigma TSB™ with Sigma Swab®				Sigma TSB™ with PurFlock® Swab			
	Control	0hr	24hr RT	24 hr 37°C	Control	0hr	24hr RT	24 hr 37°C
MSSA NCTC 13297	2.17×10^5	3.07×10^4	4.00×10^3	TNTC	2.47×10^5	2.00×10^3	1.58×10^4	TNTC
S. aureus NCTC 8350 COWAN	1.49×10^5	2.07×10^4	1.97×10^4	TNTC	3.77×10^5	1.37×10^3	$2.70\ 10^4$	TNTC
S. aureus ATCC 6358	2.67×10^5	6.03×10^4	3.03×10^4	TNTC	1.49×10^6	2.40×10^3	3.18×10^4	TNTC
Hospital Acquired MSSA isolate	3.12×10^5	7.20×10^4	1.27×10^4	TNTC	2.03×10^5	8.87×10^3	1.53×10^5	TNTC
S. aureus co-agulase posi-tive	3.56×10^5	1.03×10^4	1.95×10^4	TNTC	3.56×10^5	3.23×10^4	3.65×10^4	TNTC
S. aureus 359	2.58×10^5	4.57×10^4	1.57×10^4	TNTC	2.92×10^5	1.07×10^4	2.37×10^4	TNTC
S. aureus Ox-ford NCTC 6571	2.69×10^5	4.23×10^4	1.00×10^4	TNTC	2.69×10^5	3.33×10^4	1.50×10^4	TNTC
S. aureus 852E	3.40×10^4	1.33×10^4	2.57×10^4	TNTC	3.40×10^4	1.63×10^4	2.03×10^4	TNTC
E. coli	5.60×10^5	3.10×10^4	1.40×10^5	1.37×10^6	5.60×10^5	6.03×10^4	5.07×10^4	7.19×10^5

Figure 1. Comparison of *E.coli* (a) and MSSA 13297 (b) growth on TSA after 24 hours in Sigma TSB™ with Sigma Swab® at 37°C (Dilution 10^{-2})



Molecular confirmation

Table 2. Confirmation by PCR on BD Max™ System of MRSA results from Sigma TSB™ with 6.5% NaCl

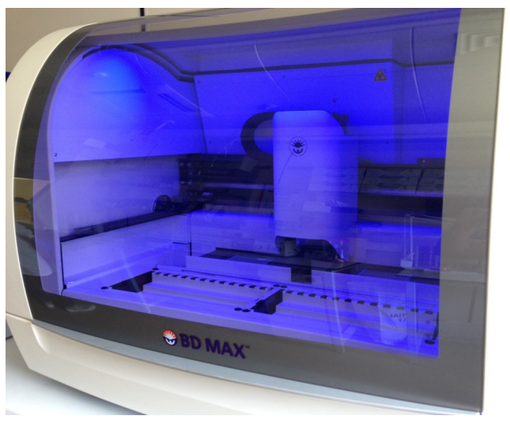
Specimen ID	Sigma TSB™ with Sigma Swab®		Sigma TSB™ with PurFlock® Swab	
	0hr	24 hr 37°C	0hr	24 hr 37°C
1	MRSA Neg	MRSA Pos	MRSA Pos	MRSA Pos
2	MRSA Neg	MRSA Pos	MRSA Pos	MRSA Pos
3	MRSA Neg	MRSA Pos	MRSA Neg	MRSA Pos
4	MRSA Neg	MRSA Pos	MRSA Pos	MRSA Pos
5	MRSA Neg	MRSA Pos	MRSA Pos	MRSA Pos
6	MRSA Neg	N/A	MRSA Pos	N/A
Negative Control		MRSA Neg		MRSA Neg

Discussion/Conclusions

The results demonstrate that when incubated overnight at 37°C, the Sigma-TSB™ with 6.5% sodium chloride (NaCl) significantly and consistently enriched all the strains of *Staphylococcus aureus*, including MRSA. There was no notable increase in the number of *E. coli* under the same conditions. By plating the enriched samples onto an appropriate chromogenic agar it would be possible to quickly detect MRSA strains.

The second part of the study demonstrated that MRSA specimens enriched in the Sigma-TSB™ tubes could be readily identified using the PCR – based tests on the BD Max™ System. There were no false positives or negatives. The two types of swab, Sigma® polyurethane foam and PurFlock® polyester flocked fibres, gave identical results for all of the enriched specimens, although the PurFlock® was also very sensitive with the time zero specimens for the BD Max™

The study has shown that Sigma TSB™ with 6.5% NaCl is a suitable device to use for hospitals wishing to implement the Dutch guideline on the laboratory detection of methicillin-resistant *Staphylococcus aureus.* It removes the need for separate processing of specimens arriving in the laboratory, in that the tubes can be directly inoculated when the specimen is taken from the patient. Positive results can be quickly and reliably confirmed on the BD Max™ PCR platform, while the results with the PurFlock® version suggest that it could be possible to test urgent specimens almost immediately following collection while still allowing the conventional culture test to proceed as normal.



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References

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- Clinical and Laboratory Standards Institute (CLSI). *Quality Control of Microbiological Transport Systems; Approved Standard- Second Edition*. CLSI document M40-A2

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Addendum to 'Evaluation of a new collection and enrichment swab device for direct testing in accordance with Dutch guideline on the laboratory detection of methicillin-resistant *Staphylococcus aureus'*

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Date: 5th May 2015

This addendum is for the additional results collected after initial poster presentation at the Scientific Spring Meeting KNVM & NVMM on 14th April 2015.

Results:

Table 1. Recovery and Enrichment of MRSA strains with Sigma-TSB™ with 6.5% NaCl. RT - Room Temp, TNTC - Too numerous to count. Results shown as CFU/ml

Strain	Sigma TSB™ with Sigma Swab®				Sigma TSB™ with PurFlock® Swab			
	Control	0hr	24hr RT.	24 hr 37°C	Control	0hr	24hr RT	24 hr 37°C
1. MRSA 22115	9.50×10^5	2.19×10^4	9.10×10^3	TNTC	9.50×10^5	4.63×10^4	1.21×10^4	TNTC
2. MRSA *Mu3* 24261	1.25×10^5	2.9×10^4	1.94×10^4	TNTC	1.25×10^4	5.93×10^4	2.13×10^4	TNTC

Discussion

The results demonstrate that when incubated overnight at 37°C, the Sigma-TSB™ with 6.5% sodium chloride (NaCl) significantly and consistently enriched both MRSA strains which support our previous findings using MSSA and S*taphylococcus aureus* strains.