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Evaluation of new Collection and Enrichment Device for MRSA

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Abstract & Introduction

Sigma-TSB™ with 6.5% NaCl is a swab-based device that combines procedures for collection and enrichment of MRSA-screening specimens. The swab is placed into a transport tube containing tryptic soy broth with 6.5% sodium chloride. Incubation at 37 °C should enrich any strains of *Staphylococcus aureus* ready for testing on chromogenic agar for MRSA.

This study was designed to assess the ability of Sigma-TSB™ to enrich strains of S. aureus (including MRSA and MSSA) in preparation for culture. In addition the compatibility of the enriched specimens with the BD Max™ System real-time PCR platform was assessed.

For culture, suspensions of various *S. aureus* (MRSA & MSSA) and one *E. coli* (for inhibition testing) strains were used to inoculate the swabs, incubated at 37° C for 24 hours before plating on Tryptic Soy Agar. For PCR, MRSA strains were inoculated onto the swabs and incubated at 37°C. Samples of the medium from 0 hour and 24 hour were placed into PCR tubes and tested on BD Max™.

All strains of *S. aureus* showed significant enrichment at 37°C, and strains tested with the BD Max™ were correctly identified.

Using Sigma-TSB™ with 6.5% NaCl as the collection device eliminates the need for a separate sub-culture step. The study shows that the device allows enrichment of *S. aureus* strains for culture, and is compatible with PCR for urgent specimens. The study also assessed the comparative performance of the medium with two types of swab, both the standard Sigma foam tip swab normally provided with the swab set, and Sigma PurFlock which is also available as an option.

An earlier presentation reported the culture results of the study for various strains of MSSA, and the results with BD Max for repeated tests of a single isolate of MRSA[1]. The study has now been extended to include multiple strains of MRSA for both the culture and molecular phases.

Methods

The performance of the new device was evaluated for two types of swab. 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 both were included in the study.

Recovery and enrichment

1. Suspensions of 8 strains of methicillin sensitive Staphylococcus aureus (MSSA), 2 strains of methicillin resistant *Staphylococcus aureus* and 1 strain of *Escherichia coli* were prepared to a concentration of 106 CFU/ml. Serial dilutions, 10^{-1}, 10^{-2}, 10^{-3}, were made. 50 µl (Sigma®) or 100 µl (PurFlock®) aliquots were dispensed into the wells of a microtitre plate for each swab to be tested, for each organism / dilution / holding combination. The inoculum amount is in accordance with CLSI M40-A2 Approved Standard[2].
2. 100µl of each suspension was inoculated onto a Tryptic Soy Agar (TSA) control plate and incubated at 37 °C for 24 hours.
3. 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).
4. After the holding times (time 0 and 24 hour) The tubes for the time 0 swabs were vortexed for 15 seconds, then using a pipette 100 µl of the liquid medium was inoculated onto an a fresh TSA plate. The plates were incubated at 37 °C for 24 hours.
5. 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

Stage 1

1. 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.
2. 5 Sigma-TSB™ with Sigma® Swab and 5 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.
3. Both types of inoculated swabs (Sigma® and PurFlock®) were incubated at 37 °C for 24 hours.
4. In addition 1 uninoculated tube from each swab type was incubated at 37 °C for 24 hours as a negative control.
5. After the holding period, all the tubes were tested by vortexing, and taking 100 µl 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.

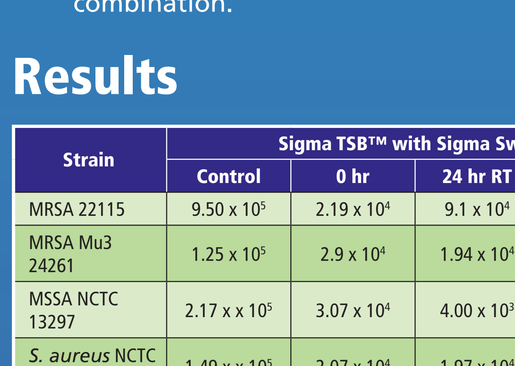
Stage 2

1. 0.5 McFarland suspensions of 24 previously isolated MRSA strains were prepared and serially diluted. 50µl aliquots of the 10^{-2} dilutions were dispensed into the wells of a microtitre plate.
2. 24 Sigma-TSB™ with Sigma® Swab and 24 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.
3. All of the inoculated swabs (Sigma® and PurFlock®) were incubated at 37 °C for 24 hours.
4. After the holding period all the tubes were tested by vortexing, and taking 100 µl of the medium by pipette and placing into a PCR tube for processing on BD Max™. All 48 tubes were tested with the BD Max™ MRSA XT panel.

Results

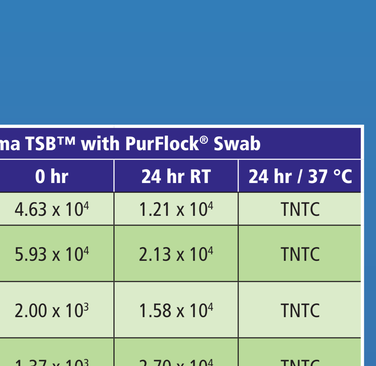
Strain	Sigma TSB™ with Sigma Swab®				Sigma TSB™ with PurFlock® Swab			
	Control	0 hr	24 hr RT	24 hr / 37 °C	Control	0 hr	24 hr RT	24 hr / 37 °C
MRSA 22115	9.50×10^5	2.19×10^4	9.1×10^4	TNTC	9.50×10^5	4.63×10^4	1.21×10^4	TNTC
MRSA Mu3 24261	1.25×10^5	2.9×10^4	1.94×10^4	TNTC	1.25×10^4	5.93×10^4	2.13×10^4	TNTC
MSSA NCTC 13297	2.17 x x 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 x x 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 x x 105	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 coagulase positive	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 Oxford 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 x 106	5.60×10^5	6.03×10^4	5.07×10^4	7.19×10^5

Table 1. Recovery and Enrichment of MSSA and MRSA using the Sigma –TSB™ with 6.5% NaCl. RT=Room Temperature, TNTC=to numerous to count. Results shown as CFU/ml



(a) *E. coli* after 24 hours at Room Temperature

(b) *MRSA* after 24 hours at Room Temperature



Molecular confirmation

Specimen ID	Sigma TSB™ with Sigma Swab®		Sigma TSB™ with PurFlock® Swab	
	Result from BD Max™ for 24 hr 37°C		Result from BD Max™ for 24 hr 37°C	
	MRSA Positive	MRSA Negative	MRSA Positive	MRSA Negative
6 tests from single isolate of MRSA	6	0	6	0
24 different patient isolates of MRSA	24	0	24	0
Negative Control	0	MRSA Neg	0	MRSA Neg

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

Discussion

The results demonstrate that when incubated overnight at 37 °C, the Sigma-TSB™ with 6.5% sodium chloride (NaCl) significantly and consistently enriched all strains of MSSA and 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.

The use of tryptic soy broth with 6.5% sodium chloride is recommended as a preliminary enrichment step for MRSA screening specimens by the Dutch guideline on the laboratory detection of methicillin-resistant *Staphylococcus aureus*.[3] Sigma TSB™ with 6.5% NaCl has been designed to combine this enrichment step with the initial specimen collection, and allowing for immediate processing by the laboratory without further handling. Where laboratories are equipped with automated processing platforms, the tubes can be loaded straight into the system, allowing for efficient throughput and reporting of MRSA status.

The study has shown that Sigma TSB™ with 6.5% NaCl is a suitable device to use for hospitals wishing to implement such a protocol for the processing of MRSA screening specimens. In addition, positive results can be quickly and reliably confirmed on the BD Max™ PCR platform. Both types of swab, whether Sigma foam–tipped, or PurFlock® polyester fibre, are suitable for use with this medium.

References

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2. Clinical and Laboratory Standards Institute (CLSI). Quality Control of Microbiological Transport Systems; Approved Standard-Second Edition. CLSI document M40-A2, 2014
3. Kluytmans-van den Bergh, M. F. Q., M. C. Vos, B. M. W. Diederen, C. M. J. E. Vandenbroucke-Grauls, A. Voss & J. A. J. W. Kluytmans, & on behalf of the Dutch working group on the laboratory detection of highly resistant microorganisms, 2014, Dutch guideline on the laboratory detection of methicillin-resistant Staphylococcus aureus, Eur J Clin Microbiol Infect Dis. 33:89 –101

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