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Comparison of ESwab and wound fiber swab specimen collection devices for use with the Xpert SA Nasal Complete assay

Running title: Molecular Detection of MRSA/MSSA with ESwabs

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ABSTRACT:

Paired nasal swab specimens were collected from patients undergoing routine MRSA screening prior to elective cardiac or orthopedic procedures. Each patient was swabbed using a traditional wound fiber liquid Stuart swab and an ESwab device, a flocked swab with a modified liquid Amies microbiology transport medium. Both specimens were tested using the Cepheid Xpert SA Nasal Complete Assay. Results demonstrated 95.5% agreement between ESwab and the FDA-cleared wound fiber swab collection device.

SHORT NOTE:

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of post-surgical infection. Several recent studies have shown that pre-operative screening with nucleic acid amplification tests and subsequent decolonization of MRSA can significantly reduce the rate of post-surgical MRSA infection (1,2). The Xpert SA Nasal Complete assay ([SA Complete] Cepheid, Sunnyvale, CA) is a molecular test approved for screening nasal specimens for the presence of methicillin-resistant and methicillin-susceptible *S. aureus* (MRSA/MSSA).

As automation of the microbiology laboratory becomes more common, the use of liquid based microbiology (LBM) specimen transport systems is also increasing. To standardize collection devices across all testing platforms, laboratories must consider validating the use of these devices with molecular tests in addition to culture-based microbiology (3). In our facility, MRSA screening is frequently performed by collecting nasal swab specimens using a Liquid Stuart BBL™ Dual CultureSwab™ ([traditional swab] BD Diagnostics, Sparks, MD) and testing is performed using the SA Nasal Complete test (one swab is broken off directly into the SA Complete Assay Elution Reagent vial). A previous analytical study indicated that ESwabs are suitable collection devices for use with the Xpert MRSA assay (Cepheid) (4). The purpose of the current study was to determine whether an ESwab™ collection device ([ESwab] Copan

Diagnostics, Murrieta, CA), a LBM bacterial transport system, could be used in lieu of a traditional swab for routine MRSA screening from patient nasal swab specimens using the SA Complete assay.

Specimens from patients undergoing MRSA/MSSA screening were collected in parallel with both a traditional swab (the FDA-cleared specimen collection device for this assay) and an ESwab. Each swab was placed in both nostrils, with the collection being made in a randomized order (at the collector's discretion). The ESwab collection device consists of a flocked swab, which is used to collect the specimen, and a vial containing 1 mL of modified liquid Amies Bacterial Transport Medium. Following collection, the swab is broken off into the transport medium, which is in turn used for inoculation of culture medium or as input for molecular assays. Upon receipt in the laboratory, one of the two traditional swabs from the dual swab collection device was used for testing with the SA Complete assay according to the manufacturer's protocol. ESwab specimens were vortexed and 200 µL (20% of the ESwab volume) was added to an SA Complete Assay Elution Reagent vial in lieu of the traditional swab; the remainder of the procedure was carried out according to the manufacturer's package insert (5).

Following testing, the performance of ESwab was determined by assessing positive and negative percent agreement using the result from the traditional swab as the gold standard. The Xpert MRSA test (performed on remaining traditional swab from dual swab collection device) was used as the discriminator for any differences observed in the MRSA result of the SA Complete assay (MSSA discrepant analysis was not performed). The MRSA test detects the same target that the SA Complete assay uses to identify MRSA strains, but does not have *S. aureus* specific primers/probes. McNemar's test was performed to determine whether the results obtained with the two collection devices were significantly different (two-tailed *p*-value <

0.05) (6,7). In addition to positive and negative percent agreement, the average Ct value (amplification cycle number where fluorescence crosses a defined threshold to become positive) for each of the four targets in the assay (SPC = specimen processing control, SPA = *S. aureus* protein A gene, *mecA* = *mecA* gene, SCC = *Staphylococcal* cassette chromosome) was compared to determine if either collection device had a significantly lower Ct value (i.e., more sensitive). The average Ct value was compared using a two-tailed student's T-test and was considered significant if the T-test returned a *p*-value of < 0.05.

Two hundred twenty-three paired specimens were obtained for the study. Seventeen specimens had invalid results that could not be resolved with traditional swabs. Five specimens were invalid with ESwab devices. One specimen was invalid with both swabs. Two specimens were excluded due to inaccurately labeled containers. The final analysis contained 198 specimens that could be compared directly with traditional and ESwab collection devices in the SA Complete assay.

The SA Complete assay provides two separate qualitative results: one for the presence of *S. aureus* (positive if the SPA target is detected) and one for the presence of methicillin resistance (positive if SPA target, *mecA*, and SCC targets are detected). Specimens collected using ESwab showed a 93.7% and 96.3% positive and negative percent agreement with traditional swab for the detection of *S. aureus* (Table 1), which is not statistically significant ($p = 1.0$). ESwab showed an 85.7% positive percent agreement and a 100% negative percent agreement for detection of methicillin resistance (Table 2). Discrepant analysis of the MRSA result was performed using the Xpert MRSA Assay with the remaining traditional swab, which was taken to be the true result. Following discrepant analysis the positive and negative percent agreement for methicillin resistance resolved to 92.3% and 100%, respectively (Table 2), which was not statistically significant ($p = 0.5$). A significant limitation of this study was the limited number of

positive MRSA specimens (n = 13). Future studies of this nature should focus on populations with an increased prevalence of MRSA.

In addition to the qualitative results, the Ct values for all of the positive staphylococcal targets (*spa*, *mecA*, and SCC) were recorded. The average Ct value was compared between the two collection devices for each target and no significant difference in Ct value was observed despite the fact that only 20% of the ESwab specimen was used as input for the molecular test, while the entire traditional swab specimen is consumed by the assay. The internal process control (SPC) consists of *Bacillus globigii* spores that are present as a dried cake in each test cartridge to ensure that lysis and thermal cycling conditions are sufficient to release and amplify *S. aureus* DNA if it is present in the specimen. Because the SPC comes in the test cartridge, the amount present in the assay should be the same with both collection devices despite the fact that only 20% of the ESwab specimen is loaded into the test. Interestingly, the average Ct value for the SPC was significantly lower (i.e., more sensitive) with the ESwab collection device specimens (p = 1.42 x 10^{-7}) (Table 3). A more detailed investigation is required to determine the exact nature of this observation. However, some possibilities include a more efficient amplification process with ESwabs, a more complete rehydration of the dried cake and better extraction of the SPC due to an increased volume of liquid being added to the test cartridge, or a decrease in the amount of inhibitors present in the specimen, as only 20% of the ESwab specimen goes into the test.

In this study the ESwab device had 66% fewer invalid results compared to traditional swabs (6 vs. 18, respectively) in the SA Complete assay with no significant difference in sensitivity and specificity. This study suggests that the ESwab collection device is at least equivalent to traditional wound fiber swabs for sample collection and analysis using the Cepheid GeneXpert SA Nasal Complete Assay.

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REFERENCES:

1) **Pofahl WE, Ramsey KM, Nobles DL, Cochran MK, Goettler C.** 2011. Importance of methicillin-resistant *Staphylococcus aureus* eradication in carriers to prevent postoperative methicillin-resistant *Staphylococcus aureus* surgical site infection. Am Surg **77:**27-31.
2) **Jog S, Cunningham R, Cooper S, Wallis M, Marchbank A, Vasco-Knight P, Jenks PJ.** 2008. Impact of preoperative screening for methicillin-resistant *Staphylococcus aureus* by real-time polymerase chain reaction in patients undergoing cardiac surgery. J Hosp Infect **69:**124-130.
3) **Borbeau PP, Ledeboer NA.** 2013. Automation in clinical microbiology. J Clin Microbiol **51:**1658-65.
4) **Silbert S, Kubasek C, Uy, D, Widen, R.** 2014. Comparison of ESwab with traditional swabs for detection of methicillin-resistant *Staphylococcus aureus* using two different walk-away commercial real-time PCR methods. J Clin Microbiol **52:** 2641-2643.
5) **Silbert S, Kubasek C, Galambo, F, Vendrone E, Widen R.** 2015. Evaluation of BD Max StaphSR and BD Max *MRSAXT* assays using ESwab-collected specimens. J Clin Microbiol **53:** 2525-2529.
6) **McNemar Q.** 1947. Note on the sampling error of the difference between correlated proportions or percentages. Psychometrika **12**:153-157.

149 **7)** **Stuart A**. 1955. A Test for Homogeneity of the Marginal Distributions in a Two-Way
150 Classification. Biometrika **42**:412-416.
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153 Table 1. Percent Agreement of *S. aureus* Target with Traditional and ESwab Collection Devices
154 in the SA Complete Assay.
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		Traditional Swab			
		S. aureus Positive	*S. aureus* Negative	*S. aureus* Invalid	TOTALS
ESwab Specimen	*S. aureus* Positive	59	5	1	65
	S. aureus Negative	4	130	16	150
	S. aureus Invalid	1	4	1	6
TOTALS		64	139	18	221
POSITIVE PERCENT AGREEMENT = 59/63 = 93.7% (95% C.I. 83.7 – 98.0)					
NEGATIVE PERCENT AGREEMENT = 130/135 = 96.3% (95% C.I. 91.1 – 98.6)					
TOTAL PERCENT AGREEMENT = 189/198 = 95.5%					

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158 Table 2. Percent Agreement of Methicillin Resistance Target with Traditional and ESwab

159 Collection Devices in the SA Complete Assay.

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		Traditional Swab			
		MecA Positive	*MecA* Negative	*MecA Invalid*	TOTALS
ESwab Specimen	*MecA* Positive	12	0	1	13
	MecA Negative	2*	184	16	202
	MecA Invalid	0	5	1	6
TOTALS		14	189	18	221
POSITIVE PERCENT AGREEMENT = 12/14 = 85.7% (95% C.I. 56.2 – 97.5)					
NEGATIVE PERCENT AGREEMENT = 184/184 = 100% (95% C.I. 97.5 – 100.0)					
TOTAL PERCENT AGREEMENT = 196/198 = 99.0%					
*One specimen negative for *S. aureus* and *MecA* with the ESwab specimen and negative for the presence of *MecA* with the Xpert MRSA assay. This represents a false positive with the traditional swab and the true positive percent agreement is likely 12/13 = 92.3% (95% C.I. 62.1 – 99.6)					

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163 Table 3. Comparison of Ct Values Obtained with Traditional and ESwab Collection Devices for

164 Each of the Four Analytes in the SA Complete Assay.

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Target	# Specimens*	Traditional Swab		ESwab		T-Test p-value (significant < 0.05)
		Average Ct	Standard Dev. of Ct	Average Ct	Standard Dev. of Ct	
SPC (Internal Control)	147*	34.64	1.90	33.64	1.20	1.42×10^{-7}
S. aureus protein A	60	25.43	4.91	25.40	5.10	0.98
MecA Gene	140	29.93	4.20	29.73	4.55	0.71
SCC Cassette	13	25.86	4.90	26.12	6.41	0.91
*Only included specimens that had an actual Ct value with both the traditional and ESwab collection devices (the SPC does not need to amplify in specimens that are positive for *S. aureus*, *MecA*, or the SCC cassette).						

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