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A Comparison of the ESwab with Traditional Swabs for the Detection of MRSA Using Two Different Walk-Away Commercial Real Time PCR Methods

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Running Title: Use of the ESwab for the Detection of MRSA

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Abstract

The ESwab (Copan Diagnostics) was evaluated as a nasopharyngeal specimen collection device to be used for MRSA detection by GeneXpert® and BD MAX™ MRSA Assays. Different MRSA strains and dilutions of each strain were tested in triplicate. The ESwab proved to be a suitable collection device for both assays tested.

Methicilin-Resistant *Staphylococcus aureus* (MRSA) is a major cause of healthcare acquired infections (1, 2). Early identification of patients with MRSA nasal carriage can be part of an effective infection prevention program (3, 4, 5, 6, 7, 8). There are commercial Real-Time PCR assays that provide MRSA results in less than a couple of hours. The Xpert MRSA® assay (Cepheid, Sunnyvale, CA), which runs exclusively on the GeneXpert® system (Cepheid, Sunnyvale, CA) and, the BD MAX™ MRSA assay (BD Diagnostics, Québec, Canada) performed on the BD MAX System™ (BD Diagnostics, Sparks, MD) are examples of these assays (9, 10, 11, 12). Both are sample in answer out tests, allowing fast results, reducing hands-on time and improving laboratory efficiency. This is a great improvement when we compare to culture based methods, which can take up to 72 hours to identify MRSA strains (9,10). Still, PCR based methods require concomitant cultures to recover organisms for epidemiological typing or for further susceptibility testing. For these reasons, sometimes the patient has to be submitted to more than one swab collection, each one to be used in a different lab test.

ESwabs (Copan Diagnostics Inc, Murrieta, CA) use a single swab liquid-based collection and transport system with a uniquely designed nylon flocked swab. In this new swab, the organism inoculum is efficiently released into 1mL of Amies liquid making it possible to perform multiple tests (PCR and culture) on the collected sample and avoiding the collection of more than one swab per patient (13, 14, 15, 16,17). The aim of this study was to evaluate and compare the performance of the ESwab and the Traditional Swab (BBL™ CultureSwab™ Liquid Stuart, BD Diagnostics, Sparks, MD), recommended by the assay manufactures, for the detection of MRSA using two different Real-Time PCR assays: the Xpert MRSA® (Cepheid) and the BD MAX™ MRSA (BD Diagnostics).

Two different MRSA strains isolated from patients attending Tampa General Hospital (TGH - Tampa, FL) were used in this study. Strains were previously characterized by strain typing at TGH, using the DiversiLab Rep-PCR instrument (bioMérieux, France). Two different clusters were identified: Cluster E and Cluster AB, both frequently isolated in patients attending TGH. Strains were first saved in the Esoteric Testing Lab Bank of Microorganisms and then, recovered in Blood Agar plates (BBL) for the tests.

An initial 0.5 MacFarland (1.5×10^8 CFU/mL) suspension of each strain was prepared in 5mL of 0.85% physiological saline, followed by seven 10 fold dilutions (1.5×10^7 to 10^1 CFU/mL) also prepared in saline. Each strain and dilution was tested in triplicate. First, 600µL of each dilution was distributed into six wells of a microtiter plate (100µL/well). Each ESwab and Traditional Swab triplicate was inoculated with 100µL of the dilution by placing the swab into one of the six wells of the prepared microtiter plate, and allowing 10 seconds for the swab to absorb the suspension. After inoculation, swabs were placed into their respective transport medium. Prior to testing, the ESwab tube was vortexed for 5 sec and a 200µL aliquot from the transport medium was transferred either to the Xpert MRSA® lysis elution buffer or to the BD MAX™ MRSA sample buffer tube. Samples were vortexed again for 5 sec before loading into a MRSA cartridge. The ESwab has a superior absorption capacity than Traditional Swabs; thus, a volume greater than 100µL would have been used if the ESwab itself was transferred directly into the assay buffer. For this reason, a 200µL aliquot from the ESwab transport medium was initially chosen to be used in this study. Traditional swabs were transferred directly into the assay buffer tube and vortexed for 5 sec before loading into a MRSA cartridge. In the end, 96 tests for each Real-Time PCR assay were performed, 48 tests using ESwabs and 48 tests using Traditional Swabs.

All results from 1.5 x 10^8 to 10^2 CFU/mL dilutions were positive for MRSA, after testing by both Real-Time PCR assays and swab types. The Real-Time PCR threshold (Ct) result values from the same dilution, but different swab types and Real-Time PCR assays were very similar to each other and, as expected, all the Ct values increased inversely proportional to the bacteria concentration. Ct values from triplicate tests were averaged and results are presented in figure 1. The dilution 1.5 x 10^1 CFU/mL from Cluster E and Cluster AB showed positive results in the three traditional swab samples tested on the BD MAX™ MRSA and in two out of the three traditional swab samples tested on the Xpert MRSA®. The same dilution showed negative results in the three ESwab samples tested on the Xpert MRSA® (Cluster E) and in one out of the three ESwab samples tested on the BD MAX™ MRSA (Cluster AB).

The ESwab transference to the ESwab medium results in a 1/10 dilution of the initial inoculums and only 1/5 of that was initially used for the Real-Time PCR assays. Therefore, to approximate the aliquot concentration to at least 1/2 of the original inoculum concentration, these negative result tests were repeated using 500µL of the ESwab liquid medium instead of 200µL. MRSA positive results were detected in all of these repeated tests (Table 1). Ultimately, the limit of detection observed from ESwab samples using 500µL of the ESwab liquid medium (1.5 x 10^1 CFU/mL) was in line with Xpert MRSA® (10 to 100 CFU/swab) and BD MAX™ MRSA (273 to 645 CFU/swab) assay analytical sensitivities previously reported by the manufacturers (20, 21).

Rapid and accurate identification of MRSA isolates is essential not only for patient care, but also for effective infection control programs to limit the spread of MRSA (1, 4, 6, 8, 18, 19). In the last few years, several commercial rapid tests for detection of MRSA directly from nasal swabs have been developed for use in clinical laboratories (9, 10, 11, 12, 18, 19). Real-Time

PCR and other molecular tests are gaining popularity as MRSA screening tests, especially because they are faster than culture methods in identifying patients who are candidates for contact precaution at the time of admission. Currently, there are two automated sample in answer out walk away Real-Time PCR assays for MRSA: the Cepheid Xpert MRSA assay performed on the GeneXpert instrument and the BD MAX MRSA Assay performed on the BD MAX instrument. These assays are validated for use only with nasal specimens taken on BBL™ CultureSwab™ Liquid Stuart (BD Diagnostiscs) or Venturi Transystem™ Swab Liquid Stuart (Copan Diagnostics) (20, 21). This means that if further investigations are required on the clinical specimen (strain typing, antibiotic susceptibility tests, or a simple repeat of the test), a second swab from the same patient will have to be collected.

Several studies have been demonstrating the superior absorption and release capacity of the ESwab comparing to Traditional Swabs (13, 14, 15, 16, 17, 22, 23, 24). The ESwab is a revolutionary concept because of its ability to offer what standard swabs cannot provide; ESwab elutes the entire sample into 1mL of transport medium, providing identical aliquots of liquid sample suspension that enable laboratories to determine and validate the optimal volume of specimen (and therefore amount of analyte) to utilize in their assay. This is the first report of the use of the ESwab as a collection device system for the two MRSA sample in answer out walk away Real-Time PCR assays. The results obtained showed that the ESwab system is a suitable sample collection device alternative for both, Xpert MRSA® and the BD MAX™ MRSA assays. Still, it is important to adjust the volume of eluted specimen to 500 µL in order to obtain similar sensitivities as the Traditional Swabs. Moreover, it is possible to perform different tests (PCR and culture) on the same collected sample, avoiding collection of more than one swab sample from the same site, per patient.

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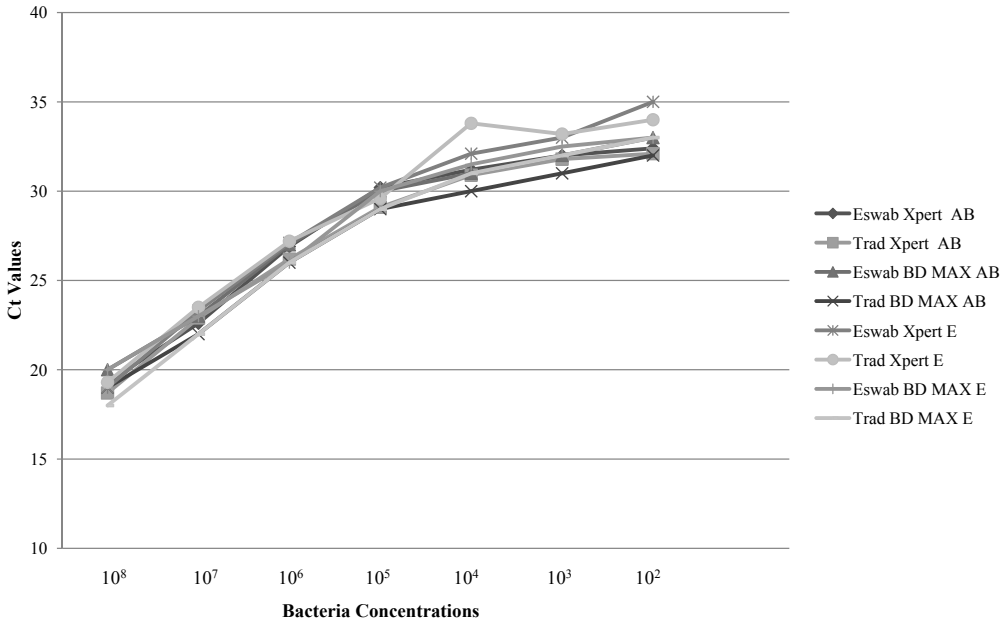
196 **Figure 1.** Real-Time PCR Ct Values from 1.5 x 10^8 to 10^2 Bacteria Dilutions



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199 **Table 1.** Real-Time PCR Ct Values of 1.5 x 10^1 Bacteria Dilution Samples

	Sample	Volume Used	Ct Values	Volume Used	Ct Values
Cluster E					
Eswab Xpert MRSA®	Sample 1	200µL	Negative	500µL	28.5
Eswab Xpert MRSA®	Sample 2	200µL	Negative	500µL	27.7
Eswab Xpert MRSA®	Sample 3	200µL	Negative	500µL	29.0
Cluster AB					
Eswab BD MAX™ MRSA	Sample 1	200µL	34.0	-	-
Eswab BD MAX™ MRSA	Sample 2	200µL	34.0	-	-
Eswab BD MAX™ MRSA	Sample 3	200µL	Negative	500µL	32.0

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