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Use of CultureSwab Plus Swabs with Amies Gel Agar with the IDI-MRSA™ Assay

J. Riley, B. Shoemaker, K. Jones, and P. Bourbeau
Geisinger Medical Center, Danville, PA. 17822

REVISED ABSTRACT

The IDI-MRSA™ assay (GeneOhm Sciences, San Diego, CA) is an FDA-cleared test for the direct detection of nasal colonization with methicillin-resistant Staphylococcus aureus (MRSA). Many institutions, including ours, selectively or broadly screen new admissions for MRSA nasal colonization to facilitate appropriate isolation procedures for MRSA carriers. Utilized in conjunction with the Smart Cycler™ instrument (Cepheid, Sunnyvale, CA), the IDI-MRSA™ assay provides rapid real-time results. The product insert specifies that specimens to be tested with the IDI-MRSA™ assay be collected with a swab with liquid Stuart media such as the BBL CultureSwab. In our laboratory, we routinely utilize the BBL CultureSwab Plus, an Amies gel swab collection device. The purpose of this study was to compare the use of the CultureSwab and the CultureSwab Plus to detect MRSA using the IDI-MRSA™ assay. The standard IDI-MRSA™ protocol used for swabs with liquid Stuart media was modified slightly for use with agar gel swabs with the addition of one extra processing step, heating at 95 +/- 2C for 2 minutes. The remainder of the processing was identical to the protocol used for swabs with liquid media. Preliminary work in our laboratory with the IDI-MRSA™ assay indicated that the sensitivity of the assay was generally between 10^2 and 10^3 CFU. For this evaluation, we prepared dilutions with intended final concentrations of 100, 500, and 1000 CFU that were used to inoculate CultureSwab swabs with liquid Stuart's media and CultureSwab Plus swabs with Amies gel agar media. A total of 6 swabs were inoculated for each isolate (2 media at 3 dilutions).120 recent clinical isolates were tested with 274 out of a possible 360 positive test results with the Culture Swab Plus swabs with Amies gel and 303 out of a possible 360 positive test results for the CultureSwab swabs with liquid Stuart's media (p=0.0003). These results suggest that the CultureSwab Plus swabs with Amies gel are an acceptable alternative to the CultureSwab swabs with liquid Stuart's media for use in the IDI-MRSA™ assay but may not be as sensitive when specimens contain lower concentrations of organisms.

MATERIALS AND METHODS

I. 120 recent unique patient clinical isolates of Staphylococcus aureus were used for this study.
II. Dilutions were made to prepare 3 inocula with targeted concentrations of 10(4), 5 x 10(3) and 10(3) per ml.
III. 0.1 ml was added to each swab. A total of 6 swabs were inoculated for each isolate (2 media at 3 dilutions) using a microtiter plate. Final targeted inocula were 100, 500, and 1000 CFU/swab.
IV. Processing. Liquid Stuart gel swabs were processed following instructions in the package insert. For the agar gel swabs, an additional step was added (indicated in black below):
   A. Remove one swab from the holder and place in the sample buffer tube. Break the swab stem off into the tube. Do this by holding the swab stem near the rim of the tube (use a kim-wipe to minimize the risk of contamination). Lift the swab a few millimeters from the bottom and bend the stem against the tube to break it. Discard the swab stem and kim-wipe. Tightly cap the sample buffer tube.
   B. Vortex at high speed for one minute.
   C. Using a sterile disposable fine-tip pipet, transfer the entire cell suspension to a yellow-capped lysis tube.
   D. Heat at 95 +/- 2C for 2 minutes.
   E. Centrifuge at high speed (>14000 x g) for 5 minutes at room temperature.
   F. Using a sterile disposable fine-tip pipet, remove the supernatant and discard. Be careful not to touch the pellet during this step.
   G. Add 50 ul of sample buffer to each lysis tube and close tightly. Use a new pipette tip for each specimen. Save remaining buffer.
   H. Vortex at high speed for five minutes.
   I. Centrifuge the lysis tubes briefly using the quick spin button on the centrifuge. Hold for 8 seconds and then release.
   J. Heat the lysis tubes at 95 +/- 2C for 2 minutes.
   K. Remove the lysis tubes and place in tube holder and maintain at 2-8C until pipetted into the Smart Cycler reaction tubes.

TABLE 1

Number of Positive Test Results for each swab type with different concentrations of inocula

Concentration	No of Positive Results[a] Both	Gel Swab	Liquid Swab	*P* value
1,000 CFU	117	0	3	NS[b]
500 CFU	98	4	16	0.007
100 CFU	40	15	29	0.03
Total	255	19	48	0.0003

[a] maximum of 120 positive results for each concentration
[b] Not statistically significant

CONCLUSIONS

- Testing of gel swabs with the IDI-MRSA™ assay requires only a minor modification of the protocol used for the liquid swabs.
- The liquid swab is more sensitive than the gel swab for detection of MRSA when lower concentration of inocula are used.
- The clinical significance of the differences in vitro sensitivity is not known. We stressed the assay/swab system by testing at inocula concentrations close to the limits of sensitivity of the IDI-MRSA™ assay.

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