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## Verification of the IDI-MRSA Assay for Detecting Methicillin-Resistant *Staphylococcus aureus* in Diverse Specimen Types in a Core Clinical Laboratory Setting

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**The IDI-MRSA assay has a sensitivity of 96% and a specificity of 96% when used to screen patients at extranasal sites. This verification study used previously unverified swabs and was undertaken in a core medical laboratory using nonmicrobiology technologists trained in sample processing, molecular laboratory work flow, and PCR practice.**

Surveillance strategies in the United States have focused primarily on identifying methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from nasal swabs. Recent data suggest, however, that the rectum and wounds are also important sites of MRSA colonization and that these sites should be screened as part of MRSA surveillance protocols (1, 5, 8). The long turnaround times and relatively low sensitivities of culture-based MRSA surveillance protocols may increase the risk of transmission to contacts (2, 7). To date, the IDI-MRSA assay remains the only PCR assay certified for MRSA screening of nasal specimens in both the United States and Canada. The purpose of this study was to expand this verification to include other specimen types and previously unverified swabs so that the IDI-MRSA assay may be implemented in areas where extranasal screening is routine. This prospective study compared the test characteristics of traditional MRSA culture to those of the IDI-MRSA assay for specimens from nonnasal sites, which were collected using an Amies gel-based swab not previously validated for the IDI-MRSA assay ([geneohm.com/english/documents/MRSA\\_CLSI\\_procedure.doc](http://geneohm.com/english/documents/MRSA_CLSI_procedure.doc)).

Surveillance swabs were collected from patients by trained nursing staff following standard operational procedures (3) and were placed into Amies gel transport medium without charcoal (Starplex Scientific Inc., Etobicoke, Ontario, Canada). The swabs were defined by source and included perineal, rectal, wound, and axillary-groin swabs and combinations of nasal plus axillary-groin-perineal swabs. Mannitol salt agar supplemented with 10 mg per liter of cefoxitin (MSA-FOX; Oxoid, Nepean, Ontario, Canada) was inoculated directly with each patient specimen prior to testing with the IDI-MRSA assay. Bundled swabs with a single label and order number (i.e., nasal plus axillary-groin-perineal swabs) were pooled onto a single MSA-FOX plate. Inoculated MSA-FOX plates were

incubated in ambient air at 35°C and examined after 18 and 36 h for yellow colonies or any other colonies resembling *S. aureus*. Identification of MRSA involved Pastorex Staph Plus agglutination (Bio-Rad, Hercules, CA) and the use of tube coagulase (Remel, Lenexa, KS) for species identification of *S. aureus* and penicillin binding protein 2a agglutination (Denka Seiken, Tokyo, Japan), and the use of CLSI oxacillin salt screen agar for the detection of methicillin resistance (3, 11).

A laboratory information system epidemiology report was generated at University Health Network/Mount Sinai Hospital Microbiology Laboratory to capture all culture-positive MRSA surveillance specimens from all sources in the previous week and to match negative samples with the specimen source and day of collection. Blinded barcoded swabs were shipped to the core laboratory at North York General Hospital, Toronto, Canada, for PCR testing.

A PCR laboratory was established within the core clinical laboratory of North York General Hospital (catchment, approximately 440,000) following CLSI recommendations (10). Certified medical laboratory technologists in the core laboratory were trained in the use of the IDI-MRSA assay. Single swabs were processed per the manufacturer's guidelines, while multiple swabs which had been pooled and inoculated onto single MSA-FOX culture plates were also pooled for screening by the IDI-MRSA assay (13). Samples that were unresolved due to the presence of inhibitors were frozen at -20°C for at least 2 h and then retested per the IDI-MRSA assay protocol ([geneohm.com/english/documents/MRSA\\_CLSI\\_procedure.doc](http://geneohm.com/english/documents/MRSA_CLSI_procedure.doc)). Further unresolved samples were diluted 1:20 and then 1:100, as necessary, until the internal control was valid for the sample.

In the case of primary culture-positive and PCR-negative samples, reassessment occurred by (i) replication of the PCR assay and (ii) determination that the MRSA isolate obtained from the original MSA-FOX culture was truly positive and not a misidentification. If samples were IDI-MRSA assay positive but culture negative, then (i) PCR lysates were reamplified; (ii) a new swab was inserted into the remaining Amies transport

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TABLE 1. Comparison of results obtained by MSA-FOX culture and the IDI-MRSA PCR assay for the detection of methicillin-resistant *Staphylococcus aureus* directly from various types of surveillance swabs

Source of swabs	No. of samples				
	Total	Culture positive, PCR positive	Culture negative, PCR negative	Culture positive, PCR negative	Culture negative, PCR positive
Axilla-groin	21	7	14	0	0
Nasal-axilla-groin-perineum	164	50	105	2	7
Perineum	26	8	14	1	3
Rectum	8	2	6	0	0
Wound	88	28	54	1	5
Total	307	95	193	4	15

medium and then processed per the standard culture protocol; (iii) the original swab was enriched in brain heart infusion broth (Oxoid, Nepean, Ontario, Canada), and the broth culture was inoculated onto secondary MSA-FOX and 5% sheep blood Columbia agar with colistin and nalidixic acid; and (iv) the patient's laboratory culture result history, from both before and after swab collection, was reviewed to identify a history of MRSA colonization or infection.

In the case of MSA-FOX culture-negative and PCR-positive results, a truly positive result was defined as (i) one where upon repeat culture of the original swab, a positive culture result was obtained by either MSA-FOX primary culture or subsequent broth enrichment, or (ii) one where the laboratory had grown MRSA from a swab from another body site that was received on the same day as the PCR-positive, culture-negative swab.

All data were entered into an Excel database (Microsoft Office Excel 2003; Microsoft Canada Co., Mississauga, Ontario, Canada). Following unblinding, discordant results were identified and repeat testing was performed as necessary. Statistical analysis was performed with Vassar stats (faculty.vassar.edu/lowry.clin1.html).

**Specimen types.** In this study, a total of 307 specimens, including 26 perineal, 8 rectal, 88 wound, 21 axillary-groin, and 164 nasal-plus-axillary-groin-perineal swabs, were tested (Table 1). All samples were received in Amies transport medium without charcoal. PCR inhibition occurred in 21 (6%) of these samples, including 1 of 21 (5%) axillary-groin swabs, 8 of 164 (5%) nasal-plus-axillary-groin-perineal swabs, 3 of 26 (12%) perineal swabs, 1 of 8 (12%) rectal swabs, and 8 of 88 (9%) wound swabs. PCR inhibition was overcome after freezing alone for 2 specimens, after 1:20 dilution for 18 specimens, and after 1:100 dilution for 1 specimen. Of the 21 unresolved samples, 1 was PCR positive and culture positive, 19 were PCR

negative and culture negative, and one was culture negative and deemed contaminated. The dilutions used to overcome inhibition did not appear to decrease the sensitivity of the assay compared to the current reference standard.

**Discordant results.** The majority of samples (288 of 307 swabs [94%]) provided concordant PCR and MSA-FOX culture results (Table 1). The four MSA-FOX culture-positive but PCR-negative discordant results were deemed falsely negative, and all four were negative upon repeat testing of the lysate. Among the 15 MSA-FOX culture-negative but PCR-positive discordant results, 7 were truly positive, while 8 discordant samples could not be rationalized with certainty, as described in Table 2. Of the seven truly positive samples, four (one nasal-plus-axillary-groin, one perineal, and two wound samples) were positive by broth enrichment culture and three (two perineal and one wound sample) had a same-day MRSA-positive patient laboratory culture history from a separate specimen.

**Assay characteristics.** The assay gave a sensitivity of 96% (95% confidence interval [95% CI], 89% to 99%) and a specificity of 93% (95% CI, 88% to 96%), using culture-based MRSA detection from primary culture screening plates as the relative gold standard. After reassessment of truly positive results, taking into account the broth enrichment results and same-day MRSA histories, the performance characteristics of the test improved, resulting in a sensitivity of 96% (95% CI, 90% to 99%) and a specificity of 96% (95% CI, 92% to 98%) (Table 2).

The implementation of a rapid, easy-to-use molecular MRSA assay which is verified for use at nonnasal sites should help to ensure that patients colonized at these sites are identified in a more timely manner than that for existing protocols (3). The weakness of the less expensive culture-based method-

TABLE 2. Causes of discordance for samples initially identified as positive by IDI-MRSA PCR and negative by primary culture

Source of swabs with discordant results	PCR positive, culture negative <sup>a</sup>	Obtained from patients defined as MRSA positive	No. of swabs			
			Broth enrichment culture positive		Same-day MRSA patient history <sup>b</sup>	
			Yes	No	Yes	No
Nasal plus axilla-groin-perineum	7	1	1	6	0	7
Perineum	3	3	1	2	2	1
Wound	5	3	2	3	1	4

<sup>a</sup> Culture negative, initial unenriched selective culture performed on MSA-FOX.

<sup>b</sup> Same-day history indicates that the patient was positive for MRSA from other specimens obtained on the same day.

ology is that during the testing period, the chance of MRSA transmission to noncolonized (roommate) contacts from unknown carriers has been modeled to be at least 30% or higher (9). Current practice in our region is to screen swabs from multiple body sites for MRSA, using culture-based methods. This study was also unique because it utilized swabs in Amies transport medium, which had not been verified previously for the IDI-MRSA assay ([geneohm.com/english/documents/MRSA\\_CLSI\\_procedure.doc](http://geneohm.com/english/documents/MRSA_CLSI_procedure.doc)).

This study provides preliminary evidence that the IDI-MRSA assay can be used for nonnasal specimens. As with previous work, the sensitivity and specificity of the assay were not 100% (13). The potential reasons for the less-than-perfect sensitivity for the IDI-MRSA assay compared to MRSA culture-based detection methods include variability in *Staphylococcus* cassette chromosome *mec* and/or *orfX* sequences that fail to be amplified in this assay (13) and the fact that primary swabs were first used to inoculate culture plates and subsequently processed for PCR. In some cases, the IDI-MRSA assay gave a discordant positive result while primary MSA-FOX culture failed to grow MRSA. This could relate to the molecular test having a higher sensitivity than the gold standard (truly positive results) (4, 13) or to nonspecific amplification leading to false-positive results. Previous work has shown that the molecular mechanisms for false-positive results with the IDI-MRSA assay are still not completely understood, but they have been postulated to involve the detection of remnant *Staphylococcus* cassette chromosome *mec* elements lacking the *mecA* gene (4). False-positive results may negatively impact health care by inappropriately placing patients into contact isolation and by exposing them to MRSA if grouping of positive patients into a cohort is practiced.

In conclusion, this study provides early evidence that the IDI-MRSA assay may be implemented on swabs from nonnasal body sites. This study also indicates that the IDI-MRSA assay still retains a high sensitivity with direct sample testing, without the requirement for initial broth enrichment of samples from rectal and wound swabs, as suggested in other studies (4, 6, 12). The IDI-MRSA assay may be implemented in a core

laboratory setting by nonmicrobiology specialist medical laboratory technologists trained in sample processing, molecular laboratory work flow issues, and the use of the IDI system.

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