Comparison of Two Versions of the IDI-MRSA Assay Using Charcoal Swabs for Prospective Nasal and Nonnasal Surveillance Samples[▽]

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An updated IDI-MRSA assay version was released to address the assay's low positive predictive value (PPV). A prospective analysis of two assay versions indicated no significant improvement in the PPV. Colonization by methicillin-resistant *Staphylococcus aureus* in 24% of patients would not have been detected if only nasal samples had been tested, as approved, by this molecular method.

Although the prevention of infections with methicillin-resistant *Staphylococcus aureus* (MRSA) in the hospital setting is extremely challenging, it has positive impacts on both the cost of care and patient outcomes (7, 10, 12). The importance of MRSA control in hospital settings was recently reemphasized by the Institute for Healthcare Improvement 5 Million Lives Campaign, which focuses on reducing unnecessary mortality (http://www.ihi.org/IHI/Programs/Campaign). One key objective of this campaign is to reduce the burden of MRSA in the health care setting. The campaign encourages a number of interventions, including the consideration of active-surveillance cultures to detect MRSA infection and colonization, as previously recommended by the Society for Healthcare Epidemiology of America guidelines (9). The authors of the guidelines believe that a sensitive and specific PCR assay may play a key role in active surveillance by providing rapid identification of colonized patients and earlier discontinuation of unnecessary infection control precautions for those proven not to be colonized.

IDI-MRSA (BD GeneOhm, San Diego, CA) is the only Food and Drug Administration-approved PCR assay for the direct detection of nasal colonization by MRSA in the United States and Canada (6, 13). The IDI-MRSA assay simultaneously detects targets in the staphylococcal cassette chromosome *mec* (SCC *mec*) and *orfX* genes specific for *S. aureus* (6). We recently undertook the successful verification of this assay as a screen for MRSA in specimens from nasal and nonnasal sites by using an Amies clear gel-based swab (5). One key concern with this assay is that although the assay has excellent sensitivity and a high negative predictive value (NPV), it appears to have a low positive predictive value (PPV) due to false positives (2, 4, 11, 13). It is possible that this low PPV may result from nonspecific amplification or the presence of remnant SCC *mec* cassettes which do not contain the *mecA* gene (4, 11). As a result of these concerns, an updated IDI-MRSA assay version (V3) with enhanced primer specificity to reduce

nonspecific amplification was put on the market in March 2006 and is identifiable by the addition of the letter "z" to the product lot number. We started to use the new version (V3) at North York General Hospital, Toronto, Canada, in expanded screening protocols in August 2006.

In this study, we compared the test characteristics of V3 (August 2006 to January 2007) with those of the previous version (V2; October 2005 to July 2006) for prospective use at North York General Hospital, a 400-bed community teaching hospital (catchment area population, approximately 440,000) with a low prevalence of MRSA. We sought to compare the two versions of the assay and, in addition, undertake the verification of an Amies gel-based charcoal swab, not included in the list of transport systems recommended by the manufacturer, for nasal, rectal, and other specimen types.

Surveillance swabs from nasal, rectal, and other sites (open chronic wounds and exit sites), if applicable, were collected from patients between October 2005 and January 2007 and placed into Amies gel-based transport medium with charcoal (Copan, Italy). Swabs were first inoculated onto MRSA-selective medium (Bio-Rad Laboratories, Marnes-la-Coquette, France) for culture (1, 8) and then processed for the IDI-MRSA PCR assay as recommended by the manufacturer (rectal and nonnasal specimens were processed as nasal specimens). The sample plates were incubated in ambient air at 35°C for 24 h. Any pink colonies were confirmed to be MRSA by using standard microbiology tests, including Pastorex Staph Plus agglutination (Bio-Rad, Hercules, CA), tube coagulase (Remel, Lenexa, KS), and penicillin binding protein 2a agglutination (Denka, Seiken, Tokyo, Japan) assays and susceptibility testing in accordance with Clinical and Laboratory Standards Institute standards (3). Samples with unresolved PCR results due to inhibition were frozen, diluted (1:20 and then, if necessary, 1:100), and retested.

Concordant PCR and culture results were accepted to be true results. PCR-negative, culture-positive results were deemed false negatives. PCR-positive, culture-negative results were reviewed further, due to the fact that molecular assays may have higher sensitivity than the "gold standard" culture comparator. These results were classified as false positives if subsequent broth enrichment cultures on both the PCR buffer and the remaining Amies transport medium were negative but

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TABLE 1. Comparison of performance of the IDI-MRSA assay V3 version (August 2006 to January 2007) to that of the V2 version
(October 2005 to July 2006) for detection of MRSA from diverse specimens using Copan charcoal swabs

Swab type(s)	Test version(s)	No. of swabs tested	No. with indicated result[a] by culture and PCR				Test characterization[b]				PCR inhibition	
			TP	TN	FN	FP	% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)	None	% (95% CI)
Nasal	V2	590	31	540	2	17	93.9 (78.4–98.9)	96.9 (95.1–98.2)	64.6 (49.4–77.4)	99.6 (98.5–99.9)	16	2.7 (1.7–4.4)
	V3	398	27	359	0	12	100 (84.5–100)	96.8 (94.3–98.2)	69.2 (52.3–82.5)	100 (98.7–100)	8	2.0 (1.0–3.9)
	Both	988	58	899	2	29	96.7 (87.5–99.4)	96.9 (95.5–97.9)	66.7 (55.7–76.2)	99.8 (99.1–99.9)	24	2.4[d] (1.6–3.6)
Rectal	V2	589	28	540	1	20	96.6 (80.4–99.8)	96.4 (94.4–97.7)	58.3 (43.3–72.1)	99.8 (98.8–99.9)	75	12.7 (10.3–15.7)
	V3	398	27	352	2	17	93.1 (75.8–98.8)	95.4 (92.6–97.2)	61.4 (45.5–75.3)	99.4 (97.7–99.9)	40	10.1 (7.5–13.4)
	Both	987	55	892	3	37	94.8 (84.7–98.7)	96.0 (94.5–97.1)	59.8 (49.0–69.7)	99.7 (98.9–99.9)	115	11.7[d] (9.8–13.8)
Other	V2	109	8	94	0	7	100 (60–100)	93 (86–97)	53 (27–78)	100 (95–100)	2	1.8 (0.5–6.4)
	V3	43	3	38	0	2	100 (31.0–100)	95.0 (81.8–99.1)	60.0 (17.0–92.7)	100 (88.6–100)	1	2.3 (0.4–12.1)
	Both	152	11	132	0	9	100 (67.9–100)	93.6 (87.9–96.9)	55.0 (32.0–76.2)	100 (96.5–100)	3	2.0 (0.7–5.6)
All	V2	1,288	67	1,174	3	44	95.7 (87.2–98.9)	96.4 (95.1–97.3)	60.4[c] (50.6–69.4)	99.7 (99.2–99.9)	93	7.2 (5.9–8.8)
	V3	839	57	749	2	31	96.6 (87.3–99.4)	96.0 (94.3–97.2)	64.8[c] (53.9–74.5)	99.7 (98.9–99.9)	49	5.8 (4.5–7.6)
Total	Both	2,127	124	1,923	5	75	96.1 (90.7–98.6)	96.2 (95.3–97.0)	62.3 (55.2–69.0)	99.7 (99.4–99.9)	142	6.7 (5.7–7.8)

[a] TP, true positive; TN, true negative; FN, false negative; FP, false positive.
[b] 95% CI, 95% confidence interval.
[c] PPV of V2 versus that of V3, $P = 0.52$.
[d] Results for nasal swabs compared to those for rectal swabs, $P < 0.0002$.

were classified as true positives if these enrichment cultures grew MRSA or if another specimen taken from the same patient on the same day was found to be MRSA culture positive.

From October 2005 to January 2007, 2,127 samples (988 nasal and 987 rectal swabs and 152 samples from other sites) from 845 patients were prospectively collected. Of the 2,127 samples, 1,923 (90%) showed concordant negative (PCR-negative and culture-negative) results (Table 1). Another 101 (4.7%) of the 2,127 samples showed concordant positive (PCR-positive and culture-positive) results. Twenty-three samples with discordant results were reclassified as true positives by the predefined criteria. Five of these 23 were positive by broth enrichment culture. Eighteen of the 23 samples were deemed to be true positives based on a culture-positive result for another specimen taken on the same day. Five samples (0.2%) gave false-negative results, and 75 (3.5%) yielded false-positive results. Thus, the overall characterization of the assay was as follows: sensitivity, 96.1%; specificity, 96.2%; PPV, 62.3%; and NPV, 99.7% (Table 1). In our hands, the false-positive rate was comparable to those demonstrated in other studies, which range from 2.9 to 5.1% (2, 4, 11).

The two versions of the assay (V2 and V3) demonstrated similar characteristics (Table 1) with little difference in sensitivity, specificity, and NPV. The PPV of V3 did not significantly improve over that of V2 (64.8 versus 60.4%; $P = 0.52$). The comparison of the two assay versions was broken down according to specimen types and demonstrated no meaningful differences (Table 1).

PCR inhibition was noted for 142 (6.7%) of 2,127 samples, and the results for all these samples were resolved completely by freezing and dilution. Overall, the rate of inhibition for rectal swabs, 115 of 987 (11.7%), was significantly higher than

that for nasal and other swabs, 27 of 1,140 (2.4%) ($P < 0.0002$) (Table 1). There was no difference in rates of PCR inhibition between V3 and V2 (Table 1).

A total of 72 (8.5%) of 845 patients were colonized with MRSA (Table 2). Of this total, 71 patients had a nasal swab taken, with 54 (76%) of the nasal swabs positive for MRSA by the IDI method. Seventy-one MRSA-positive patients also had a rectal swab taken, and a total of 54 (76%) of the rectal swabs tested positive for MRSA by the IDI method. Of the 18 swabs taken from other sites of MRSA-positive patients, a total of 10 (56%) were identified as being MRSA positive by the IDI method. An IDI MRSA screening strategy testing both nare and rectum samples would have detected 67 (96%) of 70 colonized patients. All were identified by the IDI assay when the results of all three swab sites were combined.

TABLE 2. Sensitivity of the IDI-MRSA-based screening strategy to detect MRSA-colonized patients

Swab source(s)	No. of positive swabs detected/no. of true-positive swabs[b]	% Sensitivity
Nares	54/71	76
Rectum	54/71	76
Other[a]	10/18	56
Nares and rectum	67/70[c]	96
Nares, rectum, and other	72/72	100

[a] Other sources included open wounds and line exit sites.
[b] The number of positive swabs detected is the number of samples from the site(s) specified detected as positive for MRSA by IDI-MRSA. True-positive specimens were defined as those determined to be positive by culture or those corresponding to specimens from other sites collected on the same day and found to be positive by culture.
[c] Only swabs from patients who had both rectal and nasal sites tested are included.

Despite the introduction of an updated IDI-MRSA assay version designed to reduce the number of false-positive reactions, we found that the V3 continues to be plagued by false-positive results, giving a low PPV which is particularly evident in our low-prevalence setting. Whether this problem is due to nonspecific amplification or the deletion of the *mecA* gene (with remnant SCC *mec*) needs to be further investigated. Two recent studies suggest that false-positive results from the IDI-MRSA assay are most likely due to non-*mecA*-containing SCC elements (4, 11).

Testing of nasal swabs alone appears insufficiently sensitive even with the use of molecular methodology. Of the 17 colonized patients (24%) whose nasal samples tested negative, 12 were identified by rectal swab results, 2 were identified by both rectal and other swab results, and 3 were identified by other swab results. Given these findings, our data suggest that rather than testing nasal swabs alone, consideration should be given to including the rectum as a screening site for improved active-surveillance sensitivity. There is already some evidence to suggest that this issue may be adequately addressed without the significant reagent costs of multiple-body-site testing by pooling specimens from multiple sites (2, 4).

In summary, we verified that an Amies-based charcoal swab, not recommended by the manufacturer but frequently used in microbiology labs in Canada, can be used to detect MRSA colonization with the IDI-MRSA assay with specimens from nasal and nonnasal sites and provides test characteristics comparable to those previously published (2, 4, 11). Both the recent (V3) and past (V2) versions of the IDI-MRSA assay demonstrate excellent sensitivity and high NPVs for MRSA screening of our patient population. The new version did not, however, significantly reduce the false-positive rate in our low-prevalence setting, suggesting that culture confirmation of all positive IDI-MRSA results should continue to be undertaken. Moreover, our study suggested that a screening program relying solely on nasal swabs may not be an optimal approach to screening for MRSA colonization since 24% of MRSA-colonized patients would not have been detected if only nasal swabs were included, despite the excellent analytical sensitivity of PCR technology.

REFERENCES

1. **Ben Nsira, S., M. Dupuis, and R. Leclercq.** 2006. Evaluation of MRSA Select, a new chromogenic medium for the detection of nasal carriage of methicillin-resistant *Staphylococcus aureus*. Int. J. Antimicrob. Agents **27:** 561–564.
2. **Bishop, E. J., E. A. Grabsch, S. A. Ballard, B. Mayall, S. Xie, R. Martin, and M. L. Grayson.** 2006. Concurrent analysis of nose and groin swab specimens by the IDI-MRSA PCR assay is comparable to analysis by individual-specimen PCR and routine culture assays for detection of colonization by methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **44:**2904–2908.
3. **CLSI.** 2006. Performance standards for antimicrobial susceptibility testing, M100–S16. CLSI, Wayne, PA.
4. **Desjardins, M., C. Guibord, B. Lalonde, B. Toye, and K. Ramotar.** 2006. Evaluation of the IDI-MRSA assay for detection of methicillin-resistant *Staphylococcus aureus* from nasal and rectal specimens pooled in a selective broth. J. Clin. Microbiol. **44:**1219–1223.
5. **Drews, S. J., B. M. Willey, N. Kreiswirth, M. Wang, T. Ianes, J. Mitchell, M. Latchford, A. J. McGeer, and K. C. Katz.** 2006. Verification of the IDI-MRSA assay for detecting methicillin-resistant *Staphylococcus aureus* in diverse specimen types in a core clinical laboratory setting. J. Clin. Microbiol. **44:**3794–3796.
6. **Huletsky, A., R. Giroux, V. Rossbach, M. Gagnon, M. Vaillancourt, M. Bernier, F. Gagnon, K. Truchon, M. Bastien, F. J. Picard, A. van Belkum, M. Ouellette, P. H. Roy, and M. G. Bergeron.** 2004. New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. J. Clin. Microbiol. **42:**1875–1884.
7. **Karchmer, T. B., L. J. Durbin, B. M. Simonton, and B. M. Farr.** 2002. Cost-effectiveness of active surveillance cultures and contact/droplet precautions for control of methicillin-resistant *Staphylococcus aureus*. J. Hosp. Infect. **51:**126–132.
8. **Louie, L., D. Soares, H. Meaney, M. Vearncombe, and A. E. Simor.** 2006. Evaluation of a new chromogenic medium, MRSA select, for detection of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **44:**4561–4563.
9. **Muto, C. A., J. A. Jernigan, B. E. Ostrowsky, H. M. Richet, W. R. Jarvis, J. M. Boyce, and B. M. Farr.** 2003. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and *enterococcus*. Infect. Control Hosp. Epidemiol. **24:**362–386.
10. **Noskin, G. A., R. J. Rubin, J. J. Schentag, J. Kluytmans, E. C. Hedblom, M. Smulders, E. Lapetina, and E. Gemmen.** 2005. The burden of *Staphylococcus aureus* infections on hospitals in the United States: an analysis of the 2000 and 2001 Nationwide Inpatient Sample Database. Arch. Intern. Med. **165:** 1756–1761.
11. **Oberdorfer, K., S. Pohl, M. Frey, K. Heeg, and C. Wendt.** 2006. Evaluation of a single-locus real-time polymerase chain reaction as a screening test for specific detection of methicillin-resistant *Staphylococcus aureus* in ICU patients. Eur. J. Clin. Microbiol. Infect. Dis. **25:**657–663.
12. **Rubin, R. J., C. A. Harrington, A. Poon, K. Dietrich, J. A. Greene, and A. Moiduddin.** 1999. The economic impact of *Staphylococcus aureus* infection in New York City hospitals. Emerg. Infect. Dis. **5:**9–17.
13. **Warren, D. K., R. S. Liao, L. R. Merz, M. Eveland, and W. M. Dunne, Jr.** 2004. Detection of methicillin-resistant *Staphylococcus aureus* directly from nasal swab specimens by a real-time PCR assay. J. Clin. Microbiol. **42:**5578–5581.