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Cost-Effective Frozen Master Mix Modification of a Commercial Methicillin-Resistant *Staphylococcus aureus* PCR Assay▽

Erik Munson,[1,2]* Timothy Block,[1]† Janet T. Voegeli,[1] Jeanne E. Hryciuk,[1] and Ronald F. Schell[3,4]

Wheaton Franciscan and Midwest Clinical Laboratories,[1] Wauwatosa, Wisconsin 53226[1]; College of Health Sciences, University of Wisconsin—Milwaukee, Milwaukee, Wisconsin 53201[2]; and Wisconsin State Laboratory of Hygiene[3] and Department of Medical Microbiology and Immunology,[4] University of Wisconsin, Madison, Wisconsin 53706

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The expense inherent to molecular diagnostics may be an overriding concern for a variety of clinical laboratories in the development of PCR-based methicillin-resistant *Staphylococcus aureus* (MRSA) active surveillance programs. BD GeneOhm MRSA assay master mix was reconstituted, aliquoted into SmartCycler tubes in 25-μl volumes, and frozen at −70°C. One hundred percent of archival nasal swab lysates yielded the expected PCR results when incubated in master mix frozen for 1, 2, 3, and 4 weeks. A 98.8% concordance of the final result was observed upon prospective PCR analysis of 320 clinical lysates utilizing freshly reconstituted master mix and 2-week-frozen master mix. Initial unresolved rates generated by frozen master mix and freshly reconstituted master mix differed by 1.6% ($P = 0.16$). Of 50 MRSA-positive lysates, the titers of 32 (64%) were determined to the same value upon initial tandem frozen master mix and freshly reconstituted master mix utilization; the titers of an additional 14 were determined to the same value upon repeat testing. Frozen master mix maintains potency for at least 4 weeks, facilitating detection of MRSA from nasal swab lysates, and may decrease the amount of unused reagent up to an average of 33%.

While a national surveillance program recently documented a nearly 30% increase in the intensive care unit methicillin-resistant *Staphylococcus aureus* (MRSA) infection rate from 1992 to 2003 (9), equally alarming data have been reported from surveys of hospital emergency departments. Documented purulent skin and soft tissue MRSA infection rates ranging from 15 to 74% and averaging 59% (11) suggest an additional reservoir of the organism that could significantly impact the care of hospitalized patients. These merging sources of MRSA provide impetus for health care entities to institute active surveillance programs, based largely on nasal swab specimen analysis, for detection of MRSA carriers. On a predicted basis, these programs reduce MRSA bacteremia rates by approximately 60% (15, 17). While recent data have advocated PCR-based MRSA surveillance programs based on the parameters of time to final result (2), assay sensitivity (2), and decreased incidence of transmission (4), some clinical microbiology laboratories may be apprehensive to implement PCR-based screening due to financial considerations. We hereby characterize and validate a modification of a master mix formulation protocol that not only promotes greater economy in reagent utilization but also allows for effective performance of a commercial MRSA PCR assay.

In a study protocol approved by the Wheaton Franciscan Healthcare Institutional Review Board, nasal swabs submitted for active MRSA surveillance were processed to a lysate form for the BD GeneOhm MRSA assay (BD Diagnostics, Sainte-Foy, Quebec, Canada) per the manufacturer's specifications. Kit-provided vials containing lyophilized master mix were reconstituted with 225 μl of kit-provided diluent; 25-μl aliquots were distributed into empty SmartCycler reaction tubes that were placed into a 4°C cooling block (freshly reconstituted master mix). In an experimental protocol, vials containing lyophilized master mix were reconstituted with 225 μl of kit-provided diluent; 25-μl aliquots were distributed into empty SmartCycler reaction tubes that were placed into a 4°C cooling block. Tubes were closed tightly, removed from the cooling block, and allowed to stand upright at −70°C for long-term storage protected from light (frozen master mix).

Aliquots (2.8 μl) of processed lysates were delivered to either frozen master mix tubes that were allowed to thaw for 5 min or freshly reconstituted master mix. All reaction tubes were pulse-centrifuged for 5 to 10 s and subjected to 45 cycles of PCR. Lysates were subsequently frozen at −20°C. Potency studies involved incubation of MRSA-positive/negative archived lysates in both freshly reconstituted master mix and frozen master mix that had been stored for 1, 2, 3, or 4 weeks at −70°C. A prospective clinical study analyzed 322 nasal swab lysates in tandem using freshly reconstituted master mix and 2-week-frozen master mix. All MRSA-positive lysates were subsequently titrated separately in freshly reconstituted and frozen master mix. Optimal conditions for preanalytical handling of frozen master mix tubes were determined by performance of MRSA PCR on previously determined low-titer (≤10) and high-titer (≥1,000) lysates utilizing frozen master mix tubes acclimated for 30 or 60 min at 4°C or room temperature conditions.

Those lysates demonstrating internal control amplification inhibition (unresolved results) were thawed and repeat tested within 24 h. Consensus results generated by PCR from freshly reconstituted master mix and frozen master mix constituted reference results. The significance test of proportions (13) was

* Corresponding author. Mailing address: Wheaton Franciscan Laboratory, 11020 West Plank Court, Suite 100, Wauwatosa, WI 53226. Phone: (414) 256-1479. Fax: (414) 256-5566. E-mail: Erik.Munson@wfhc.org.

† Present address: St. Joseph's Hospital, West Bend, WI 53095.

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TABLE 1. Resolution of discrepancies generated by MRSA PCR from incubation of 322 clinical nasal swab specimen lysates in freshly reconstituted master mix or experimental frozen master mix stored at −70°C

Specimen no.	PCR result for master mix				Final interpretation
	Initial		Repeat		
	Frozen	Freshly reconstituted	Frozen	Freshly reconstituted	
1	Unresolved	−	Unresolved	−	Unresolved result from frozen master mix
2	+	−	+	−	Confirmation of discrepancies
3	−	+	−	+	Confirmation of discrepancies
4	−	+	−	+	Confirmation of discrepancies
5	−	+	+	−	Unable to replicate either initial PCR result; exclusion from data analysis
6	−	+	+	−	Unable to replicate either initial PCR result; exclusion from data analysis
7	−	+	−	−	Unable to replicate initial PCR result from freshly reconstituted master mix
8	−	+	−	−	Unable to replicate initial PCR result from freshly reconstituted master mix
9	−	+	−	−	Unable to replicate initial PCR result from freshly reconstituted master mix
10	−	+	−	−	Unable to replicate initial PCR result from freshly reconstituted master mix
11	−	+	−	−	Unable to replicate initial PCR result from freshly reconstituted master mix
12	−	+	−	−	Unable to replicate initial PCR result from freshly reconstituted master mix

used to determine if differences in rates of a positive PCR result or unresolved specimen were significant. The alpha level was set at 0.05; all *P* values are two tailed.

Analysis of 21 previously positive/negative MRSA lysates yielded 100% concordance when incubated in master mix that had been frozen for 1, 2, 3, and 4 weeks. During the prospective phase of the evaluation, 92.5% of lysates incubated in freshly reconstituted master mix yielded concordant results to aliquots tandemly incubated in 2-week-frozen master mix. Repeat testing of initial unresolved lysates resulted in a concordance rate of 96.3%. Ten of 12 discordant results were adjudicated upon further analysis (including 3 confirmed discrepancies) (Table 1), bringing the final concordance rate to 98.8% in evaluable isolates. Although spontaneous *mecA* deletions have been shown to occur in MRSA (5–7), the discrepancies and result alterations illustrated in Table 1 (specimens 5 to 12) more likely indicate Poisson effect-related sampling differences at the lower limit of molecular detection (18). Twenty percent of the MRSA-positive lysates in our prospective study exhibited a titer of 1 (data not illustrated).

No significant differences were observed in the percentages of positive and unresolved results derived from lysates incubated in freshly reconstituted master mix versus those incubated in frozen master mix (Table 2). All MRSA-positive lysates ($n = 50$) titrated within a single $\log_{10}$ dilution, with 46 (92.0%) titrated to an equal value after discrepancy analysis. Acclimation of frozen master mix at room temperature had a negative effect on master mix potency, particularly upon extended-room-temperature acclimation (Table 3). This was most evident in lysates containing low-level target DNA and was attributable to internal control nonamplification.

Recent reports suggest that molecular diagnostics may be an appropriate alternative to culture for MRSA active surveillance. Boyce and Havill (2) reported a 9.4% increased detection rate of MRSA colonization by PCR over that derived from the CHROMagar MRSA assay ($P = 0.04$), with mean 25.8- and 6.7-h reductions in times to final negative and positive results, respectively. Past systems of modeling nosocomial MRSA transmission suggested that a reduction in time to final positive MRSA result would reduce both rate of transmission (12) and the number of patient isolation days (1). Raboud et al. (12) further predicted that replacement of a culture-based MRSA screening test with one based on PCR would significantly reduce the rate of MRSA transmission ($P = 0.002$).

TABLE 2. MRSA PCR-positive and unresolved rates derived from incubation of clinical nasal swab specimen lysates in fresh master mix or frozen master mix stored at −70°C

Master mix performance characteristic	Rate (%) of PCR results:		
	Positive	Unresolved	
		Initial	Final
Freshly reconstituted	16.3[a]	1.2[b]	0.0[c]
Frozen	15.9	2.8	0.3

[a] $P = 0.91$ versus frozen.
[b] $P = 0.16$ versus frozen.
[c] $P = 0.32$ versus frozen.

TABLE 3. Impact of preanalytical handling of experimental frozen master mix on subsequent MRSA PCR results from low-titer, high-titer, and mock MRSA nasal swab specimen lysates

Acclimation environment and interval	% of specimens with expected result		
	High-titer lysates ($n = 12$)	Low-titer lysates ($n = 12$)	Mock lysates ($n = 24$)
Room temp			
30 min	100.0	91.7	83.3[c]
60 min	100.0	33.3[a]	16.7[d]
4°C			
30 min	100.0	100.0	100.0
60 min	100.0	83.3[b]	100.0

[a] Eight unresolved results.
[b] One unresolved result and one negative result.
[c] Four unresolved results.
[d] Twenty unresolved results.

TABLE 4. Hypothetical matrix relating lysate batch size to the potential for discard of freshly reconstituted master mix under current BD GeneOhm MRSA assay specifications

Lysate batch size (size with controls included)	No. (%) of unused aliquots following reconstitution[a]		
	8 aliquots/vial	9 aliquots/vial	10 aliquots/vial
1 (3)	5 (62.5)	6 (66.7)	7 (70.0)
2 (4)	4 (50.0)	5 (55.6)	6 (60.0)
3 (5)	3 (37.5)	4 (44.4)	5 (50.0)
4 (6)	2 (25.0)	3 (33.3)	4 (40.0)
5 (7)	1 (12.5)	2 (22.2)	3 (30.0)
6 (8)	0 (0.0)	1 (11.1)	2 (20.0)
7 (9)	7 (43.8)	0 (0.0)	1 (10.0)
8 (10)	6 (37.5)	8 (44.4)	0 (0.0)
9 (11)	5 (31.3)	7 (38.9)	9 (45.0)
10 (12)	4 (25.0)	6 (33.3)	8 (40.0)
11 (13)	3 (18.8)	5 (27.8)	7 (35.0)
12 (14)	2 (12.5)	4 (22.2)	6 (30.0)
13 (15)	1 (6.3)	3 (16.7)	5 (25.0)
14 (16)	0 (0.0)	2 (11.1)	4 (20.0)

[a] Mandated controls were added to the batch size prior to the calculation of unused reaction tubes. The matrix assumes the presence of one 16-thermocycler block in a laboratory. The mean percentages of unused aliquots per random distribution batch size were 24.4, 29.6, and 33.5%, respectively, for 8, 9, and 10 aliquots per master mix vial.

Indeed, in a report on MRSA active surveillance in a critical care unit, Cunningham et al. (4) noted a reduction of nosocomial MRSA transmission rate from 13.9/1,000 patient days to 4.9/1,000 patient days ($P < 0.05$) upon conversion of a culture-based screening method to one based on PCR.

Despite these data, the cost of PCR technology may be prohibitive to a number of health care institutions. Conterno et al. (3) questioned the cost-effectiveness of PCR-based MRSA screening in locales of low prevalence. Wernitz et al. (14) caution laboratories to explore the role that diagnosis-related group reimbursement may play in the overall economics of a PCR-based screening program. Relating this issue of economy to a specific commercial MRSA PCR assay (from a technical standpoint), the BD GeneOhm MRSA assay provides lyophilized master mix in approximate 25-cm^3 volumes in individual vials and directs the user to reconstitute each vial with 225 μl of kit-provided diluent. Although the final reconstitution volume far exceeds 200 μl (approximating 250 μl), it is suggested by the manufacturer that eight 25-μl aliquots are available for PCR assays involving patient specimens and appropriate positive and negative controls.

Because freshly reconstituted master mix is stable for just 3 h in the original vial at 2 to 8°C per package insert, laboratories may be unable to utilize all potential aliquots for PCR testing. Table 4 provides a hypothetical matrix of the percentage of prepared master mix that could go unused under conditions of an 8-, 9-, or 10-aliquot yield per lyophilized vial. This scenario assumes a single 16-thermocycler block housed in a laboratory. As an example, when eight potential aliquots are yielded from reconstitution of a lyophilized master mix vial for a patient lysate batch size of 3 (with controls bringing the run size to 5), three of the eight aliquots (37.5%) may be unused. If it is further assumed that laboratories contribute to active surveillance in a real-time fashion, then a random distribution of batch sizes may be realized over a continuum. Based on these assumptions, on average 24.4% of master mix would go unused if eight aliquots were consistently generated upon lyophilized master mix reconstitution. That figure would increase to 33.5% if 10 aliquots were consistently generated from lyophilized master mix reconstitution.

With these rather substantial percentages of potential reagent waste, laboratories may be forced to batch patient lysates for PCR, thus not fully taking advantage of the real-time capacity of the assay and subsequently defeating some of the purpose of MRSA active surveillance. We hypothesized that reconstituting master mix as recommended and freezing it at −70°C would maximize reagent use without compromising the performance of the BD GeneOhm MRSA assay. Kofler and Klausegger (10) described an in-house PCR for detection of human cytomegalovirus from clinical specimens that utilized preformulated aliquots of master mix stored in 200-μl thin-walled tubes at −20°C. Hoorfar et al. (8) showed that reaction mixtures specific for *Salmonella enterica* 5′ nuclease (TaqMan) PCR were stable for 3 months when stored in microwell plates at −20°C. Finally, West and Sawyer (16) reported that aliquots of preformulated master mix from four real-time PCR assays, including a TaqMan assay detecting mammalian mitochondrial DNA, could be stored at −70°C without deleteriously affecting performance.

Our data contribute a commercially available real-time MRSA PCR assay to the list of molecular diagnostic assays that can use frozen master mix for routine testing. Amid concern that a freeze-thaw cycle would deleteriously affect the potency of the master mix, we demonstrated that all MRSA-positive lysates titrated within a single $\log_{10}$ dilution, with 92% titrated to the same value after discrepancy analysis. The titer of three lysates in freshly reconstituted master mix was just 1 $\log_{10}$ higher than titers derived from testing with frozen master mix. Similarly, incubation of one lysate in frozen master mix resulted in a 1-$\log_{10}$ increase in titer over that generated from incubation in freshly reconstituted master mix. Kofler and Klausegger (10) hypothesized that frozen master mix may actually gain some degree of potency during a freeze-thaw cycle as 97.6% and 90.5% of clinical lysates were positive for cytomegalovirus after being tested with frozen and freshly reconstituted master mix, respectively. Finally, West and Sawyer (16) reported that the performance of multiple real-time PCR assays did not deteriorate after master mixes were subjected to six freeze-thaw cycles.

In conclusion, kit-provided lyophilized master mix that has undergone reconstitution and subsequent prolonged storage at −70°C retains sufficient potency for routine clinical performance of the BD GeneOhm MRSA assay. Such formulation of master mix results in cost-effective kit utilization, which may allow smaller-volume laboratories to more advantageously utilize PCR technology for a real-time contribution to MRSA active surveillance.

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REFERENCES

1. **Bootsma, M. C. J., O. Diekmann, and M. J. M. Bonten.** 2006. Controlling methicillin-resistant *Staphylococcus aureus*: quantifying the effects of interventions and rapid diagnostic testing. Proc. Natl. Acad. Sci. USA **103:**5620–5625.

2. **Boyce, J. M., and N. L. Havill.** 2008. Comparison of BD GeneOhm methicillin-resistant *Staphylococcus aureus* (MRSA) PCR versus the CHROMagar MRSA assay for screening patients for the presence of MRSA strains. J. Clin. Microbiol. **46:**350–351.
3. **Conterno, L. O., J. Shymanski, K. Ramotar, B. Toye, C. van Walraven, D. Coyle, and V. R. Roth.** 2007. Real-time polymerase chain reaction detection of methicillin-resistant *Staphylococcus aureus*: impact on nosocomial transmission and costs. Infect. Control Hosp. Epidemiol. **28:**1134–1141.
4. **Cunningham, R., P. Jenks, J. Northwood, M. Wallis, S. Ferguson, and S. Hunt.** 2007. Effect on MRSA transmission of rapid PCR testing of patients admitted to critical care. J. Hosp. Infect. **65:**24–28.
5. **Deplano, A., P. T. Tassios, Y. Glupczynski, E. Godfroid, and M. J. Struelens.** 2000. In vivo deletion of the methicillin resistance *mec* region from the chromosome of *Staphylococcus aureus* strains. J. Antimicrob. Chemother. **46:**617–620.
6. **Donnio, P.-Y., F. Février, P. Bifani, M. Dehem, C. Kervégant, N. Wilhelm, A.-L. Gautier-Lerestif, N. Lafforgue, M. Cormier, the MR-MSSA Study Group of the Collège de Bactériologie-Virologie-Hygiène des Hôpitaux de France, and A. Le Coustumier.** 2007. Molecular and epidemiological evidence for spread of multiresistant methicillin-susceptible *Staphylococcus aureus* strains in hospitals. Antimicrob. Agents Chemother. **51:**4342–4350.
7. **Hiramatsu, K., E. Suzuki, H. Takayama, Y. Katayama, and T. Yokota.** 1990. Role of penicillinase plasmids in the stability of the *mecA* gene in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **34:**600–604.
8. **Hoorfar, J., P. Ahrens, and P. Rådström.** 2000. Automated 5′ nuclease PCR assay for identification of *Salmonella enterica*. J. Clin. Microbiol. **38:**3429–3435.
9. **Klevens, R. M., J. R. Edwards, F. C. Tenover, L. C. McDonald, T. Horan, R. Gaynes, and the National Nosocomial Infections Surveillance System.** 2006. Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* in intensive care units in US hospitals, 1992–2003. Clin. Infect. Dis. **42:**389–391.
10. **Kofler, B., and A. Klausegger.** 1999. Simplified PCR set-up using a frozen preformulated mix for the detection of cytomegalovirus. Diagn. Microbiol. Infect. Dis. **34:**33–35.
11. **Moran, G. J., A. Krishnadasan, R. J. Gorwitz, G. E. Fosheim, L. K. McDougal, R. B. Carey, and D. A. Talan.** 2006. Methicillin-resistant *S. aureus* infections in the emergency department. N. Engl. J. Med. **355:**666–674.
12. **Raboud, J., R. Saskin, A. Simor, M. Loeb, K. Green, D. E. Low, and A. McGeer.** 2005. Modeling transmission of methicillin-resistant *Staphylococcus aureus* among patients admitted to a hospital. Infect. Control Hosp. Epidemiol. **26:**607–615.
13. **Triola, M. F.** 1992. Elementary statistics, 5th ed. Addison Wesley, Boston, MA.
14. **Wernitz, M. H., S. Keck, S. Swidsinski, S. Schulz, and S. K. Veit.** 2005. Cost analysis of a hospital-wide selective screening programme for methicillin-resistant *Staphylococcus aureus* (MRSA) carriers in the context of diagnosis related groups (DRG) payment. Clin. Microbiol. Infect. **11:**466–471.
15. **Wernitz, M. H., S. Swidsinski, K. Weist, D. Sohr, W. Witte, K. P. Franke, D. Roloff, H. Ruden, and S. K. Veit.** 2005. Effectiveness of a hospital-wide selective screening programme for methicillin-resistant *Staphylococcus aureus* (MRSA) carriers at hospital admission to prevent hospital-acquired MRSA infections. Clin. Microbiol. Infect. **11:**457–465.
16. **West, D. M., and J. Sawyer.** 2006. Freezing complete polymerase chain reaction master mix reagents for routine molecular diagnostics. J. Vet. Diagn. Investig. **18:**580–582.
17. **West, T. E., C. Guerry, M. Hiott, N. Morrow, K. Ward, and C. D. Salgado.** 2006. Effect of targeted surveillance for control of methicillin-resistant *Staphylococcus aureus* in a community hospital system. Infect. Control Hosp. Epidemiol. **27:**233–238.
18. **Wolk, D. M., E. Picton, D. Johnson, T. Davis, P. Pancholi, C. C. Ginocchio, S. Finegold, D. F. Welch, M. de Boer, D. Fuller, M. C. Solomon, B. Rogers, M. S. Mehta, and L. R. Peterson.** 2009. Multicenter evaluation of the Cepheid Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) test as a rapid screening method for detection of MRSA in nares. J. Clin. Microbiol. **47:**758–764.