

Evaluation of Penicillin Binding Protein 2a Latex Agglutination Assay for Identification of Methicillin-Resistant *Staphylococcus aureus* Directly from Blood Cultures

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The penicillin binding protein 2a (PBP2a) latex agglutination test using a blood culture pellet was compared to the oxacillin screen agar method using isolated colonies. For blood cultures positive for *Staphylococcus aureus* ($n = 70$), the direct PBP2a test was 18% sensitive and 100% specific. The PBP2a test shows poor sensitivity when used directly with positive blood cultures.

The gene product of *mecA*, an altered penicillin binding protein (PBP2a), is the hallmark of methicillin resistance in staphylococci. The PBP2a latex agglutination test (Oxoid, Hampshire, United Kingdom) is a 20-min phenotypic test that detects PBP2a in isolated colonies (4). The PBP2a assay is faster and less complicated than PCR for *mecA* and has been shown to be more sensitive than other phenotypic methods, such as the use of oxacillin screen agar (2, 5, 7). Other investigators have demonstrated satisfactory performance of the PBP2a assay using isolated colonies (6, 8).

Conventional work flow for positive blood cultures includes subculture and overnight incubation to obtain isolated colonies. These colonies are then used to test for methicillin resistance by one of the methods mentioned above. Rapid reporting of identification and susceptibility results has been associated with improved outcomes (1, 4). Two groups reported acceptable performance of the PBP2a assay used directly with positive blood cultures, which reduces the time to result by 24 to 48 h (T. Yamazumi, I. Furuta, T. Maeno, Y. Tsubakimoto, and M. A. Pfaller, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol., abstr. C-99, 2002; L. A. Bassiwa and D. Craft, 103rd Gen. Meet. Am. Soc. Microbiol., abstr. C-86, 2003). The present study sought to confirm these results by using the ESP (TREK Diagnostic Systems, Cleveland, Ohio) blood culture system.

Isolates tested by the direct PBP2a assay were those previously determined to be *Staphylococcus aureus* by direct testing methods routinely used in the laboratory. Isolates giving a positive signal in the ESP blood culture system were subjected to Gram staining, and those identified as gram-positive cocci in clusters underwent a direct tube coagulase test. The tube coagulase test was performed using 5 drops of blood culture broth. The test was read after 4 h of incubation at 35°C, and a positive result indicated the presence of *S. aureus*. Seventy such isolates from different patients were obtained over a 4-month period.

These 70 isolates were subsequently seeded into blood cul-

ture bottles, and the bottles were pulled from the ESP instrument when the isolates gave a positive signal. An aliquot of blood culture broth was added to a 7-ml serum separator Vacutainer tube (BD, Franklin Lakes, N.J.). The tube was centrifuged at $1,300 \times g$ for 10 min. The supernatant was discarded, and the bacterial pellet was used as the inoculum for the PBP2a test. Direct testing specifically for susceptibility results by using this preparation has been described previously (3). From this point, the PBP2a assay was performed according to manufacturer instructions. The direct PBP2a test results were compared to those of the oxacillin screen agar (BBL, Cockeysville, Md.) method performed with isolated colonies. Quality control (with strains ATCC 29213 and ATCC 43300 as negative and positive controls, respectively) was performed each day of testing. PCR for the *mecA* gene was not performed; the oxacillin screen agar method was considered to provide the definitive result.

Of the 70 blood cultures positive for *S. aureus*, 44 had methicillin-resistant *S. aureus* and 26 had methicillin-susceptible *S. aureus* as determined by the oxacillin screen agar method using isolated colonies. The PBP2a assay used directly with positive blood cultures yielded the following results: 18% sensitivity, 100% specificity, 100% positive predictive value, and 42% negative predictive value. The PBP2a assay used directly with positive blood cultures lacked sensitivity, as only 8 of 44 methicillin-resistant *S. aureus* isolates were identified as methicillin resistant. Despite excellent specificity, the direct PBP2a assay yields unsatisfactory performance.

These results contradict the previous direct PBP2a assay results (100% sensitivity and 86% specificity for *S. aureus*) that Yamazumi et al. obtained using BACTEC (Beckton Dickinson, Sparks, Md.)-tested blood cultures (Yamazumi et al., Abstr. 102nd Gen. Meet. Am. Soc. Microbiol.). Procedures for test performance for the present study and that of Yamazumi et al. were similar, and contradictory results are puzzling. However, use of the PBP2a test by these investigators subsequent to presentation of the abstract has yielded poor sensitivity, and the test is not currently in use (M. Pfaller, personal communication). Bassiwa and Craft reported direct PBP2a results of 96% sensitivity and 80% specificity for *S. aureus* with the use of BACTEC-tested blood cultures (Bassiwa and Craft, 103rd

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Gen. Meet. Am. Soc. Microbiol.). However, their procedure utilized multiple additional washes and centrifugation steps and yielded poor specificity.

The extreme variability in performance of the PBP2a assay used directly with blood cultures as reported in these three studies underscores the importance of performing an in-house validation of any assay used in a manner not consistent with the instructions on the package insert. The variety of media and assays warrants an evaluation of specific combinations of these components rather than dependence on work performed by other investigators. Furthermore, while rapid identification and susceptibility testing may be associated with improved outcomes, quality must not be sacrificed in pursuit of rapid results.

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