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Unacceptable Performance and the Lack of Reproducibility Results in the Report of Colorimetric Methods for Early Detection of Vancomycin and Oxacillin Resistance in *Staphylococcus aureus*

In a recent paper by Coban and colleagues (6), two colorimetric methods for early detection of vancomycin and oxacillin resistance in *Staphylococcus aureus* were reported. The authors state that the results were compared by calculating essential agreement, absolute agreement, and minor, major, and very major errors, as recommended by the FDA (10). However, in the cited version of the FDA document, these terms were replaced by categorical agreement and minor, major, and very major discrepancy (10). The authors noted that all tests were performed in the duplicate (6, 10, 14). However, the authors did not report the results of duplicate testing that is necessary for the reproducibility evaluation. In fact, the cited FDA document describes a separate reproducibility (i.e., ≥95%) evaluation step with a triplicate testing (10). The aim of the FDA document is premarket evaluation of new methods which may not guarantee the validity of performance, and even the literature that assays the validity of performance should be read critically (13). In parallel to this view, the *Journal of Clinical Microbiology*'s editorial policy advocates Standards for Reporting of Diagnostic Accuracy (STARD) guidance in the description of diagnostic microbiology assays and comparison of assay performance (1a, 4, 15). However, to our knowledge, a study design in compliance with STARD criteria in the field of microbiology does not exist in the literature to date, possibly because this evidence-based initiative is both new and not easy to comply with. Additionally, the authors report lower-than-90% essential agreement for both methods, except in the nitrate reductase assay for oxacillin testing, and state that results were in concordance with the standard method (6). Contrarily, this is an unacceptable performance according to the criteria described in the cited FDA document (10).

In conclusion, the report lacks the reproducibility evaluation, and whether this affects the final performance metrics cannot be known. In addition, the report does not show an acceptable performance of essential agreement, except in the nitrate reductase assay for oxacillin testing. Therefore, this research note should be considered a report of colorimetric methods with preliminary results that need reproducibility testing before acceptable validation through a study design as recommended by the FDA scheme or STARD initiative. It should also be noted that there are founding and preceding milestone articles that either contributed to the development of or directly used the oxidation-reduction color indicators and nitrate reductase assays in susceptibility testing of microorganisms, none of which are cited by the authors (1, 2, 3, 5, 7, 12, 16–18). It should be underlined that the use of the rapid colorimetric method for MIC testing is not new and is already available as FDA-approved and patented technology in certain nonautomated and automated commercial susceptibility systems (9, 16). The authors stated that such an automated system is expensive, which may refer to only the original capital investment for the initial technology acquisition purchase, but the reagent rental option usually also exists (8). In fact, it has been shown that the use of approved and standardized automated systems in routine rapid susceptibility testing can be cost-effective, not to mention the added values of computerized expert systems for data analysis and interpretation (9).

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Authors' Reply

We thank I. C. Acuner and C. Eroglu for their interest in our article. We will try to answer their concerns.

We proposed these two colorimetric methods for early detection of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Staphylococcus aureus* strains. These methods determine the susceptibility and nonsusceptibility of strains with 100% accuracy. Only one vancomycin-resistant *Staphylococcus aureus* strain is defined as intermediate resistant instead of resistant. As is mentioned in the text, some FDA-approved systems are not able to discriminate these resistant strains either. We believe that our proposed methods perform well as "rapid tests." These tests, which give results in 5 to 6 h, were compared to broth microdilution, which gives results after 18 to 24 h, using FDA methodology, even though these are only "rapid-screening" tests. We did not perform all the tests required by the FDA for industry because we do not intend to develop these methods as "commercial systems." However, to answer Acuner and Eroglu's concern, we repeated all of the tests which agreed 100%, so these tests do not have reproducibility problems. Some references, including some of the references proposed by I. C. Acuner and C. Eroglu, had to be deleted when the manuscript was rewritten as a Note.

We hope that one day all laboratories in the world will be able to use "cost-effective commercial systems" and there will be no need to use easy and cheap in-house tests. But until then, we believe that these methods may be used by several laboratories, especially in the developing world.

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