Reliability of Pasco MIC System for Use in Detection of Resistant Streptococcus pneumoniae

The article by Nolte et al. (3) implied that Pasco MIC panels are not reliable in the detection of resistant strains of Streptococcus pneumoniae. These findings are contrary to data from a recent study (5) which was submitted as part of a Food and Drug Administration (FDA) 510(k) application that was approved on January 17, 1995. Therefore, it is important to understand the variations in the experimental design of these two studies that led to such different findings.

The article by Nolte et al. outlines test methods which deviate from those described in the Pasco package insert (4). These deviations are as follows,

(i) The organism inoculum was prepared incorrectly in diluent (water) with Tween rather than the MIC blood supplement. The Pasco package insert indicated that one should prepare the inoculum of fastidious, slowly growing organisms by the turbidity method and then should “prepare the subsequent dilution of the organism suspension in 12.5 ml of an appropriate supplemental diluent.” National Committee for Clinical Laboratory Standards (NCCLS) document M7-A3, referenced in the package insert (1), described appropriate supplements to be used for specific fastidious or slowly growing organisms.

(ii) The Pasco package insert described a one-step inoculation procedure which simultaneously provided for inoculation of the test organism and supplementation of the panel. Instead, 100 μl of the MIC blood supplement was added to each of the wells of the panel, effectively diluting by 50% the Mueller-Hinton broth. The resulting nutritionally deficient medium likely accounted for those pneumococcal strains which “failed to grow sufficiently for MIC determination.”

It is our belief that these changes in methodology have led to misleading conclusions regarding the ability of the Pasco panels to detect pneumococcal resistance. The Pasco panels have undergone some minor modifications to better comply with the latest NCCLS recommendations (2). However, these modifications would not account for the difference in the data obtained in the Nolte et al. study and that obtained in the independent laboratory study.

Deviation from the methodologies described in package inserts for commercial products may critically impact carefully formulated test systems. Acceptance of the FDA 510(k) substantiates that the Pasco system is a reliable system for use in the detection of resistant strains of S. pneumoniae.

REFERENCES

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Authors’ Reply
We thank Ms. Dillon for her interest and comments on our study of commercially available broth microdilution panels for detection of penicillin-resistant pneumococci (2). It is her contention that deviations from methods described in the Pasco package insert (3) accounted for the fact that 86% of the test strains failed to grow in Pasco panels when the Difco MIC blood supplement (10% lysed horse blood [LHB]) in distilled water, product 6661-42-7) was used. The method we described for these panels was an attempt to resolve an apparent discrepancy in the instructions contained in that package insert.

The package insert indicated that the inoculum for fastidious, slowly growing organisms should be prepared by using the turbidity method and that the subsequent dilution should be prepared in an appropriate supplemental diluent. NCCLS document M7-A3 (1) was given as a reference. The package insert also described a one-step inoculation procedure which simultaneously provided for inoculation of the test organism and supplementation of the broth. If the instructions in the package insert were followed, the final LHB concentration would have been 0.5% rather than the 2 to 5% recommended by NCCLS. In order to achieve a final concentration of LHB within the recommended range, we chose to add 100 μl of the MIC blood supplement to each of the wells. This addition reduced the concentrations of penicillin and Mueller-Hinton broth by one-half. We agree with Ms. Dillon that the resulting nutritionally deficient medium may have accounted for the poor growth of the test strains. Support for this hypothesis was provided in our study. Supplementation of the panel with 100 μl of 5% LHB prepared in Mueller-Hinton broth rather than water allowed for good growth of all the test strains.

The current package insert (4) describes inoculum preparation using 12.5 ml of SP blood supplement (product 6875-42-0) and indicates that the final concentration of the LHB will be approximately 2.5%. Apparently the LHB supplement has been modified to more closely approximate the NCCLS recommendations. We are pleased to learn that the recent modifications made by the manufacturer have significantly improved the ability of this test system to support the growth of pneumococci and to accurately detect penicillin-resistant strains.

REFERENCES

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Ed. Note: The letters by Dillon and Nolte underscore the profound effects of subtle variations in testing protocols. The use of
Difco LHB supplement in the original study effectively diluted the Mueller-Hinton broth to a concentration that would not support the growth of many strains of S. pneumoniae. With appropriate modifications of the supplement, that difficulty has been eliminated, as documented in the recently published study by Tenover et al. (reference 5 in Dillon’s letter). Laboratorians must use care when published studies are used for selection of diagnostic tests. All system parameters, including the testing medium and supplements, must remain constant for the comparison to be valid.