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Reevaluation of Positivity Cutoff Values for the Pneumococcal Urinary Antigen Detection Assay

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To improve the clinical diagnosis of pneumococcal infection in bacteremic and nonbacteremic community-acquired pneumonia (CAP), a Luminex technology-based multiplex urinary antigen detection (UAD) diagnostic assay was developed and validated. The UAD assay is a limit assay based on defined positivity cutoff limits and can simultaneously detect 13 different serotypes of *Streptococcus pneumoniae* by capturing serotype-specific *S. pneumoniae* polysaccharides (PnPSs) excreted in human urine. UAD assay validation and clinical validation of the corresponding positivity cutoff values were described in a previous publication in this journal (1).

This assay was originally developed for use in the Community-Acquired Pneumonia Immunization Trial in Adults (CAPiTA) study (2) (adults ≥65 years of age). After the completion of sample testing in support of CAPiTA and study 6115A1-4007 (Distribution of PCV 13 Serotype *Streptococcus pneumoniae* in Adults 50 Years and Older Presenting to Select U.S. Hospitals with Radiographically Confirmed Community-Acquired Pneumonia) (3), a critical component used in the UAD assay was received by an outside supplier and, as part of our laboratory's standard practice, was qualified for use in the UAD assay, passing all prospectively set acceptance criteria. This new reagent was used in the UAD assay for a number of epidemiological studies to study the burden of the 13 serotypes covered by Prevnar 13 in subjects with community-acquired pneumonia. Upon review of the interim UAD results generated in support of the U.S. study 1147, it was noted that the percent positivity for serotype 5 was higher than expected for the U.S. population and higher than what was observed for study 6115A1-4007, which led to a laboratory investigation of the UAD assay performance. The investigation was conducted, and it was determined that all assay controls showed consistent assay performance. However, we performed a number of additional tests that led to the finding that the new reagent did slightly affect the positivity cutoff values for 3 serotypes (5, 14, and 23F), leading to higher positivity rates. Given these results and to ensure UAD assay data comparability to older and future study data, the positivity cutoffs needed upwards adjustment to address the reagent change. The positivity cutoff values were reevaluated using similar procedures and statistical parameters previously used for the validation of the UAD assay as described in reference 1. This evaluation confirmed that adjustments to the positivity cutoff value were required for serotypes 5, 14, and 23F with the new reagent. The new cutoffs are 2.5 to 5.0 PnPS U/ml, 1.7 to 4.7 PnPS U/ml, and 8.0 to 19.1 PnPS U/ml for serotypes 5, 14, and 23F, respectively. The revised positivity cutoff values were used in the reanalysis of the raw UAD data from all affected studies.

Since reagent changes will be required in the future, an additional level of control requiring the testing of 400 healthy control urine samples in addition to all other qualification tests was implemented. The testing of such controls will also be implemented in situations where samples are obtained from countries not previously eval-

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uated by the UAD assay. In this case, the control samples will be procured from those countries to ensure that the UAD assay positivity thresholds are acceptable for specimens from those countries. These additional assay controls will continue to ensure consistent assay performance over time.

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