Analytical Validation of a Rapid Molecular Test for Semi-Quantitative Detection of Bacterial Pathogens and Antibiotic Resistance Genes in Urine

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Background: Phenotypic antibiotic susceptibility testing is the standard guide for antibiotic therapy, but it is slow and often leads to management with empiric therapy using broad-spectrum antibiotics. Rapid molecular tests detect pathogen and resistance genes but have not demonstrated the ability to comprehensively predict phenotypic resistance for Gram-negative infections.

Material/Methods: We developed a PCR test, the Acuitas® AMR Gene Panel u5.47 (RUO), for semi-quantitative detection of E. coli, K. pneumoniae, P. aeruginosa, P. mirabilis and E. faecalis plus 47 antibiotic resistance genes which can be used in conjunction with statistical algorithms of the Acuitas Lighthouse Software to predict phenotypic resistance to 13 antibiotics commonly prescribed for these pathogens. Test sample is primary urine specimens or spiked organisms in urine.

Results: Lower limit of detection for each pathogen is approximately 1000 organisms per milliliter of urine based on parallel culture plate counts. The test accurately detected pathogens with semi-quantitation over 10⁴ to 10⁶ organisms per milliliter of urine. No species cross-reactivity was detected with 39 other pathogens commonly associated with urinary tract infections, although cross-reactivity is expected for some species of Shigella, Klebsiella varicola and Proteus vulgaris with the assays for identification of E. coli, K. pneumoniae and P. mirabilis, respectively. The Acuitas Lighthouse Software accurately predicted antibiotic resistance for 35 highly resistant FDA/CDC isolates across 13 antibiotics with excellent positive predictive values. Prediction accuracy for P. mirabilis was subsequently improved by adding several previously excluded mechanisms into the Acuitas Lighthouse Software.

Conclusions: Acuitas AMR Gene Panel u5.47 (RUO) rapidly detects a comprehensive set of pathogens and resistance genes in Gram-negative bacilli with high sensitivity and specificity without unexpected cross-reactivity. Test results can be used to predict phenotypic resistance when analyzed by the statistical prediction algorithms of the Acuitas Lighthouse Software. Rapid and accurate recognition of antibiotic resistance mechanisms can improve antibiotic use and outcomes in patients with Gram-negative bacterial infections.