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A Rapid Indirect Enzyme-Linked Immunosorbent Assay for Identification of *Acinetobacter* spp. from Cultured Isolates

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Abstract:

Background: *Acinetobacter* spp. are responsible for up to 10% of cases of ventilator-associated pneumonia (VAP). Culturing delays identification, which raises the medical risks for critically ill patients because of the high prevalence of multiple drug resistance in this pathogen. Clinicians need a more rapid, reliable assay for identification of these important pathogens.

Methods: Polyclonal chicken IgY antibodies were developed against surface protein antigens from 7 individual isolates of *Acinetobacter* spp. The antibodies were used in an indirect ELISA capable of detecting live or chemically-fixed *Acinetobacter* spp. from isolated colonies. The ELISA was tested using the antigen-producing strains and 13 additional *Acinetobacter* spp. strains. Specificity testing included 30 non-*Acinetobacter* isolates: 16 non-fermenters, one *Haemophilus influenzae*, and the remainder *Enterobacteriaceae*. Each sample was analyzed with and without primary antibody to determine the relative background contribution to the overall signal. A positive was defined as a signal above the sum of the mean background plus 3 standard deviations. Bacteria were resuspended in PBS and added to a 96-well microtiter plate. Following binding, the wells were washed and blocked. Dilute anti-*Acinetobacter* polyclonal chicken IgY antibody was added to the wells. After washing, bound IgY was detected with peroxidase-linked goat anti-chicken-IgY secondary antibody. TMB substrate conversion was used to detect relative levels of peroxidase activity. The concentrations and incubation times for bacterial immobilization, primary anti-*Acinetobacter* antibody, and secondary anti-chicken IgY antibody were optimized.

Results: After optimization, the assay could be performed in less than 4 hours, including sample preparation. ELISA was positive for 15 of 20 *Acinetobacter* strains, and positive on 2 of the 30 non-*Acinetobacter* strains.

Conclusion: A 4-hour ELISA assay for *Acinetobacter* spp. demonstrated 75% sensitivity for 20 *Acinetobacter* spp. isolates. Specificity of the assay was 93% for a collection of 30 non-*Acinetobacter* GN isolates. Further development will be required to achieve full clinical utility.

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ASM Member (or who has submitted an application) : Steve Metzger