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Differentiation of *tox*-bearing *Corynebacterium* species directly from clinical specimens

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Statement of the Problem: Toxigenic strains of *Corynebacterium diphtheriae*, C. *ulcerans* and C. *pseudotuberculosis* are capable of eliciting diphtheria toxin that causes symptoms of diphtheria, a vaccine-preventable disease. Historically, diagnosis of tox-bearing *Corynebacterium* species and verification of toxin production required an isolate. The purpose of this study was to validate a new triplex real-time PCR assay to both detect the tox gene and differentiate C. *diphtheriae* from the 2 other tox-bearing *Corynebacterium* species directly from clinical specimens.

Methodology & Theoretical Orientation: The triplex assays detect the *Corynebacterium tox* gene, C. *diphtheriae* rpoB, and the rpoB ortholog in C. *ulcerans* and C. *pseudotuberculosis*. A total of 101 archived clinical specimens (throat and nasal swabs) from suspected diphtheria cases, 20 *Corynebacterium* spp. and 15 other respiratory pathogen isolates were used to examine sensitivity and specificity of the triplex assay. Comprehensive in *silico* analysis of the oligo sequences was performed to confirm specificity. When available, results were compared to previous culture, CDC singleplex tox real-time PCR, and toxin production results. Positivity was determined with a Ct less than 40.

Findings: The triplex assay demonstrated an LOD of 10 genomic copies, 10X more sensitive than the current singleplex CDC assay. No cross-reactivity was found with 15 respiratory pathogens, including other *Corynebacterium* spp. Three specimens that tested negative with the current CDC assay were found to harbor the *tox* gene using the triplex assay, 2 of which were confirmed as C. *diphtheriae*.

Conclusion & Significance: The new triplex assay successfully differentiates C. *diphtheriae* from other toxigenic *Corynebacterium* species directly from clinical specimens and is more sensitive than the current CDC assay. Although a bacterial isolate is needed to confirm toxin production, when an isolate is not available, this assay can be used to identify a potentially toxigenic strain of *Corynebacterium* in clinical specimens. Further analysis will be conducted to determine if this assay is a good surrogate for identifying truly toxigenic strains.

Biography

Katherine Bowden received her BS in Microbiology and PhD in Genetics from the University of Georgia. Over the past 4 years, she has led numerous projects under the scope of molecular diagnostics in the Pertussis and Diphtheria Lab at the Centers for Disease Control and Prevention. These projects include real-time PCR method validation, analysis of molecular epidemiology of pertussis epidemics, and development of a whole genome Multi Locus Sequence Typing (wgMLST) for pertussis. Additionally, she has coordinated numerous international trainings to build in-country capacity for both pertussis and diphtheria diagnostics in Latin American and Caribbean nations.

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Notes:

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