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DEVELOPMENT AND CLINICAL EVALUATION OF RAPID REAL-TIME RT-PCR ASSAYS FOR DETECTION OF INFLUENZA A AND B VIRUSES

Luke T. Daum, James P. Chambers, and Gerald W. Fischer

Influenza viruses type A (H3N2 and H1N1) and B are the most prevalently circulating human strains. However, an increase in confirmed cases of high pathogenic H5N1 in humans has raised concerns of a pandemic underscoring the need for rapid, point of contact detection. In this study, we describe development/evaluation of highly sensitive and specific real-time RT-PCR (rRT-PCR) assays for type, i.e., influenza A and B, and subtype, i.e., H1, H3, and H5 specific assays and subsequent evaluation using: 1) archived viral reference strains, 2) human cultures, and 3) primary (throat swab/nasal wash) clinical specimens. Type A and B assays detected all 16 (H1-H16) influenza A and both circulating B lineages (Yamagata and Victoria), respectively. Compared to 'gold standard' culture confirmation, 180/180 shell vial cultures (100%) were correctly typed and subtyped in research blinded fashion using real-time RT-PCR analysis described in this report. Furthermore, RT-PCR analysis of 167 uncultured, primary specimens revealed an overall specificity of 100% (no cross-hybridization) and sensitivity of 90.4% (151/167 uncultured specimens) from archived (1999-2006) uncultured samples compared to subsequent confirmation of these samples by culture. These influenza primer and probes have been adapted for use in an optimized, all-inclusive thermostable reagent blend and can be utilized on several real-time PCR thermocyclers including field-deployable instruments. Using the H5-specific assay, the optimized reagent blend was stable at ambient temperature for 30 days and capable of detecting < 10 viral copies. These assays could offer significant utility for rapid, point of care screening arising from a pandemic influenza outbreak.