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Real-Time RT-PCR Detection of Influenza Virus Within Symptomatic and Asymptomatic Family Members

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BACKGROUND: The 2007-08 influenza season was the worst in three years and included a total of 71 influenza-associated pediatric deaths. Increased morbidity and mortality were mostly attributed to mismatch between contemporary circulating influenza A (H3N2) and B viruses and vaccine strain components. Additionally, H1N1 Oseltamivir resistance and continued H5N1 persistence in humans underscores the need for rapid, point-of-care influenza typing and subtyping detection. This prospective (2007-08) family study compared the sensitivities of traditional culture, RAPID immunoassay (Remel X/pect) and a recently developed PrimeMix real-time RT-PCR (rRT-PCR) System for detecting influenza virus from: 1) symptomatic pediatric patients and 2) asymptomatic or mildly infected family members. **METHODS:** A total of 100 pediatric (index) patients who met the clinical case criteria for influenza infection and 126 ancillary family members were enrolled in the study. Nasal wash specimens were obtained and subjected to commercial antigen testing, traditional culture and type/subtype-specific rRT-PCR analysis. Nucleotide sequencing was performed on all influenza-positive samples. **RESULTS:** Of the total samples evaluated (N=226;100 index,126 family contacts), 66 (29%) tested positive for influenza virus (45 H3N2, 2 H1N1 and 19 B) according to rRT-PCR. rRT-PCR detected influenza virus from symptomatic pediatric index patients and asymptomatic/symptomatic family members that were not detected by culture or rapid antigen testing. **CONCLUSIONS:** The influenza assays described here can type (A or B) and subtype (H5,H3,H1) influenza from original specimens in 2 hours, are adapted for use in an optimized, thermostable reagent blend and can be utilized on several real-time PCR thermocyclers including field-deployable instruments. These assays could offer utility for point-of-care routine screening or during a pandemic influenza outbreak.