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Detection and Molecular Characterization of Clinical Influenza A and B Viruses from Original Nasal Wash Specimens Preserved in PrimeStore™

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Background: Widespread emergence of influenza drift variants among contemporary circulating human viruses prompted a change in all three vaccine components for the upcoming 2008/09 season. Increased morbidity and mortality during the 2007/08 season included 72 influenza-associated pediatric deaths and continued drug resistance (oseltamivir and adamantadine) within circulating strains. Successful nucleic acid-based influenza diagnostics are contingent upon sample collection strategies that procure high quality RNA for downstream detection and characterization. PrimeStore™ is a recently developed sample collection solution for lysing, stabilizing and preserving nucleic acids from samples including nasal wash or throat swab specimens at room temperature or above for 30 days. A prospective (2007/08) clinical study was conducted which included nasal wash specimens from: 1) symptomatic pediatric patients and 2) asymptomatic or symptomatic family members. Influenza virus was detected from nasal wash specimens collected in PrimeStore™ and Viral Transport Media (VTM) by real-time RT-PCR (rRT-PCR) and traditional culture, respectively. **Methods:** A total of 100 pediatric (index) patients who met the clinical case criteria for influenza infection and 126 family contacts were enrolled in the study. Nasal washings were placed into PrimeStore™ and Universal Viral Transport Media (Becton-Dickinson) and analyzed by rRT-PCR or culture analysis, respectively. Additional genetic characterization of selected clinical samples preserved in PrimeStore™ was performed using standard RT-PCR and nucleotide sequencing of the hemagglutinin (HA), neuraminidase (NA) and matrix (MA) viral surface proteins. **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) by rRT-PCR. Real-time RT-PCR from nasal washings preserved in PrimeStore™ detected influenza virus (5 Flu A and 2 Flu B) that were not detected by culture. Phylogenetic analysis of influenza A and B HA genes exhibited drifting compared to the 07/08 vaccine strains and revealed a higher genetic homology to the 2008/09 Brisbane vaccine strains. Some genetic differences in viruses were noted among family members, particularly in influenza A (H3N2) strains. Furthermore, MA analysis revealed adamantane resistance in all influenza A H3N2 strains but sensitivity in both H1N1 viruses. **Conclusions:** Influenza RNA obtained from specimens collected in PrimeStore™ is stable and preserved for molecular diagnostic applications as evident by real-time detection and full-length DNA gene sequencing. Real-time RT-PCR is highly sensitive and specific compared to traditional culture methods. Archived samples preserved in PrimeStore™ can be utilized for future genetic characterization of larger genome segments, vaccine development, or other molecular-based detection methods. PrimeStore™ may be advantageous for routine point-of-care collection or during a pandemic influenza outbreak.