DEVELOPMENT OF A REAL TIME REVERSE-TRANSCRIPTION PCR (RRT-PCR) ASSAY FOR DETECTION OF INFLUENZA A H1N1 2009 FROM CLINICAL RESPIRATORY SPECIMENS

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Background: In March 2009, a pandemic Influenza A virus (2009 H1N1) emerged in North America and spread worldwide, causing more than 9,500 deaths. This 2009 H1N1 strain is a reassortant, containing a swine hemagglutinin (HA) from North America, a neuraminidase (NA) and matrix (MA) from swine European strains and other human and avian origin genes. **Aims**: A newly developed H1-09 subtype specific and Internal Positive control (IPC) assay were developed, optimized and utilized in a retrospective clinical study. The results were compared to the CDC rRT-PCR and rapid antigen test. **Methods**: A PrimeMix RRT-PCR assay targeting the 2009 H1 hemagglutinin gene was developed from multiple sequence alignments of progenitor strains in GenBank. Additionally, an IPC assay was developed targeting a non-specific RNA in a clinical collection medium (PrimeStore) for monitoring specimens from collection-to-detection. A retrospective clinical study utilized nasal washings collected from two Texas DoD facilities. **Results**: The optimized H1-09 PrimeMix assay showed high sensitivity ($10^{-1.0}$ $TCID_{50}$/mL, equivalent to 1-10 viral copies) and specificity to a reference panel of viruses and bacteria. In the retrospective study, PrimeMix H1-09, FluA and FluB assays identified 79/80 samples compared to CDC Swine rRT-PCR Panel results. FDA-approved Rapid Antigen testing exhibited the lowest sensitivity and specificity among influenza detection methods. **Conclusions**: The PrimeMix H1-09 and IPC assays are highly sensitive and specific, exhibiting equivalent results compared to other detection methods. Furthermore, the IPC assay in PrimeStore Collection medium is a novel approach for monitoring specimen integrity from patient collection-to-detection.