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A Rapid, Simplified Collection-to-Detection System for Typing and Subtyping Influenza Viruses Using Real-Time RT-PCR

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Influenza type A (H3N2 and H1N1) and B viruses 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 collection and detection. In this study, we describe development of a: 1) novel respiratory specimen collection solution and 2) highly sensitive and specific set of real-time RT-PCR (rRT-PCR) assays for type, i.e., influenza A and B, and subtype, i.e., H1, H3, and H5 detection of influenza viruses from clinical specimens. The sample collection reagent was developed and optimized for procuring high quality nucleic acids from clinical or environmental specimens, inactivating potentially infectious biological pathogens for safe transport of specimens, and stabilizing and preserving released RNA/DNA preventing hydrolysis/nuclease degradation for extended periods at ambient temperatures. 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. The RT-PCR reagents are all-inclusive thus reducing possible contamination and abolishing the need for extensive reagent pipetting and 'master mix' preparation. The type A and B rRT-PCR assays detected all 16 (H1-H16) influenza A subtypes and both circulating B lineages (Yamagata and Victoria), respectively. In a family study, these assays detected influenza A from throat swabs and nasal washes and were more specific than antigen detection kits. The described specimen collection solution and stabilized influenza rRT-PCR assays could be useful for environmental, epidemiologic or point-of-care screening or during a pandemic.