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Plasmid-based Rescue of Influenza A Virus Directly from Swab Specimens

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Epidemics of influenza A virus affect 15-60 million people in United States annually, and these infections cause significant morbidity and mortality. Many subtypes of influenza A, which are differentiated on the basis of their hemagglutinin (H1-H16) and neuraminidase (N1-N9) surface glycoproteins, circulate in a variety of other species including birds. Infrequently, novel influenza A viruses emerge from such reservoirs and give rise to pandemics, in which morbidity and mortality are dramatically increased. The development of plasmid-based reverse-genetic technology, to engineer a negative-sense segmented RNA genome and recover infectious influenza A virus, has revolutionized basic research and shows great promise for vaccine production. Current reverse-genetic systems use restriction endonuclease digestion and ligation to create clones for each gene segment. To accelerate the rescue of new influenza A viruses for research and vaccine production, we modified the reverse-genetic plasmid pHH21, so as to allow recombination-based cloning of the entire influenza A genome, amplified using a multi-segment RT-PCR (M-RTPCR) strategy that we previously developed to simultaneously amplify all eight genomic segments in a single reaction. We used M-RTPCR to amplify the genome of a recent human H3N2 virus (A/NY/238/2005) directly from a clinical swab specimen, and then cloned the amplicons into our recombination-based reverse-genetic plasmids. Clones containing each A/NY/238/2005 gene segment were identified, and their nucleotide sequence was compared to the consensus sequence of the viral RNA quasi-species. Three synonymous substitutions and one nonsynonymous mutation were found in the eight clones containing the ~13.5-kb genome. The clone with the potentially deleterious mutation was replaced with a sister clone, and rA/NY238/05 was rescued. The data show that the combination of M-RTPCR and recombination-based cloning confers sensitivity, speed, fidelity, and flexibility to the rescue of any subtype of influenza A virus, without the need for in vitro propagation.