2011 ECCMID-ICC Meeting Abstract
May 7-10, Milan, Italy

Rapid killing of influenza virus and RNA preservation from samples held at elevated temperatures for extended periods

L.T. Daum*, S.A. Worthy, Y.W. Choi, G.W. Fischer (San Antonio, Columbus, OH, US)

Background: Sensitive and specific molecular detection is an important part of pathogen identification and epidemiological surveillance during pandemics and epidemics. Commercial collection and transport media, commonly referred as VTM, that maintain viability of organisms in specimens increase both infectious disease risk and RNA/DNA degradation by nucleases, oxidation and hydrolysis. A specifically designed molecular transport medium (MTM) for nucleic acid testing (NAT) has been utilized to preserve labile influenza RNA and nucleic acids from other pathogens.

Specific Aim: We report the use of a MTM to inactive/kill high titers of influenza A/Vietnam/1203/2004 (H5N1) and pandemic A/Mexico/4108/2009 (H1N1) virus. Additionally, long-term preservation of released viral RNA for 62 days at ambient temperature and 37°C was evaluated by real-time RT-PCR.

Methods: For viral killing studies, collection swabs were loaded with 0.1 mL H5N1 (1.5x107 TCID50/mL) or viral storage buffer (negative controls), placed into MTM and held for 10, 30, or 60 minutes at ambient temperature. The samples were cultured using MDCK cells for 96 hours and visually examined for CPE with total TCID50 determined. Preservation of H5N1 and H1N1 virus was analyzed using real-time RT-PCR at 0, 1, 2, 5, 7, 14, 30, and 62 days post-inoculation. Additional RT-PCR amplification of large segments was performed to analyze integrity of preserved influenza H1N1 RNA. For this experiment, aliquots were placed into MTM, incubated at 37°C for 2 weeks and tested by RT-PCR. Results: High pathogenic H5N1 influenza virus placed into MTM resulted in no detectable, viable virus and was equivalent to negative controls. RNA from H5N1 and H1N1 viruses preserved in MTM for up to 62 days at ambient temperature were detectable by real-time RT-PCR analysis with only minimal degradation of target signal. Additionally, influenza virus RNA was preserved for at least 2 weeks in MTM at 37°C using real-time PCR detection.

Conclusion: MTM rapidly kills microbes by lysing lipid membranes, and destroying proteins and enzymes (including nucleases). Further, MTM stabilizes and preserves the released nucleic acids from viruses and collected microbes. The ability to safely collect and ship clinical specimens at ambient temperature and detect viral RNA with NAT weeks later could be a valuable asset for tracking and surveillance of pandemic influenza (H5N1, H1N1/09) viruses or other emerging pathogens.