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Assessment of RNA*Sound* RNA Sampling Card for the preservation of influenza virus RNA (#154)

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Shipping of influenza viruses or their RNA is normally done on dry ice, but this is expensive and requires special packaging and shipping conditions. Therefore it would be useful to develop alternative methods for shipping RNA at ambient temperatures. We evaluated the RNA*Sound* Sampling Card (FortiusBio LLC, San Diego, CA) for the purposes of RNA preservation. Ten microliters of influenza virus sample was applied to two small push-out perforated discs of filter paper that are impregnated with a lysis buffer. Live viruses are inactivated upon application onto the card and RNA recovery is easily completed by adding the discs to RNase-free water in a microtube and vortexing.

We applied either influenza virus A(H1N1pdm09) or B cell culture isolate supernatants or their respective pre-extracted RNA onto separate cards. The cards were then stored at either room temperature (18-22°C) or in the fridge (2-8°C) for 7, 14 or 28 days. Stability of virus/RNA on cards was compared with the same samples stored at -80°C for the same period of time. Eluted RNA was assessed for stability by real-time RT-PCR and Sanger sequencing.

RNA recovered from viruses applied to the cards, regardless of storage temperature or duration of storage, was found to be highly stable with cycle threshold (Ct) values being identical or differing by only 1 Ct unit in real-time RT-PCR assays compared to RNA recovered from control virus isolates stored at -80°C. However, when we added pre-extracted RNA to the cards, there was a greater difference in Ct values (Δ Ct 3-4) compared to RNA stored at -80°C. Nevertheless, Sanger sequencing was successful when either virus isolates or pre-extracted RNA were added to the cards and stored at either temperatures for all time durations.

Further investigation is ongoing to determine if RNA preservation with RNA*Sound* is superior to ordinary filter paper.