Effect of swab type, collection media, and storage on the detection of influenza a virus in porcine nasal secretions

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Clinical samples from humans and animals require collection systems that are easy to use and will safely stabilize and preserve microbial RNA/DNA during transportation from distant collection sites to diagnostic centers. An influenza (flu) detection study was conducted to compare the performance of commonly used rayon tipped swab with liquid Stuart's transport media (RTLS) and flocked swabs with PrimeStore Molecular Transport Medium™ for collecting and transporting nasal secretions from pigs experimentally infected with H1N1 influenza A virus.

The animal phase of this study was conducted in accordance with the approval of the Institutional Care and Use Committee of the University of Minnesota. Twenty two 3-week-old swine from a herd free of flu, *Mycoplasma hyopneumoniae* and PRRSV were randomly assigned to 2 treatment groups. Ten swine per treatment group were inoculated 2 ml intratracheally and 2 ml intranasally with $10^{5.5}$ $TCID_{50}$/mL of influenza A/Swine/IL/02450/2008 (H1N1). Two swine were sham inoculated and served as negative controls. Nasal swabs were taken from each pig at 1, 3, and 5 days post-inoculation (dpi) using both the RTLS and PrimeStore MTM™ collection systems. RTLS swabs were tested immediately after collection for flu by virus isolation (VI) on MDCK cells. If virus isolation attempts were negative, the RTLS swabs were tested for flu by matrix gene RRT-PCR. PrimeStore MTM™ swabs were held for 2 weeks at 4°C and frozen for 6 weeks at -20°C prior to testing for flu by RRT-PCR. VI was not performed on PrimeStore MTM™ swabs because the transport solution is designed to safely inactivate viruses and bacteria and preserve the released RNA and DNA. Flu detection results were compared for both media types.

A total of 66 specimens were obtained and placed in each collection medium. **Both the RTLS and PrimeStore MTM™ performed similarly, with 100% agreement in flu detection results** for each pig at 1and 3dpi. Thirty-seven pigs were positive for flu by VI on RTLS collected nasal swabs and PCR on PrimeStore MTM™ collected nasal swabs. Seven pigs at 1dpi and two pigs at 3dpi were negative for flu on both RTLS nasal swabs by VI and PCR and also on PrimeStore MTM™ swabs by PCR. All RTLS flu VI positive nasal swabs were quantified by virus titration and therefore it was determined that RRT-PCR detected influenza virus from swabs collected in PrimeStore™ MTM at levels as low as 56 $TCID_{50}$/mL of virus (C_T value 31.29). The only differences in flu detection performance were found on 5dpi, where 2 RTLS nasal swabs were negative for flu by both VI and PCR, but were positive for flu in PrimeStore™ medium by PCR.

PrimeStore Molecular Transport Medium™ provides a suitable, safe, collection, handling, and preservation system that can be used to detect flu in porcine nasal secretions. PrimeStore™ even detected flu RNA in samples from pigs at 5 dpi, a time when shedding is decreased or non-detectable by VI or PCR using RTLS swabs. A larger field study to compare pecimen collection systems from naturally infected pigs is recommended and will commence in Summer/Fall 2010.