Swine Health Information Center

Improved Oral Fluids PCR Sensitivity Call for Proposals

The mission of the Swine Health Information Center is to protect and enhance the health of the US swine herd through coordinated global disease monitoring, analysis of swine health data and targeted research investments that minimize the impact of future disease threats.

Oral fluid samples are widely used in swine surveillance for PRRSV, Influenza A virus in Swine (IAV-S), and *Mycoplasma hyopneumoniae* nucleic acids on U.S. farms and have shown the potential for use in Foreign Animal Disease (FAD) surveillance, as well. To date, more than 27 pig pathogens have been shown to produce detectable levels of nucleic acid in oral fluids.

Recent unpublished, observational research indicated oral fluids (as collected by rope as an aggregate sample) could be a good sample for rapid detection of African swine fever virus (ASFv). In a negative cohort study, no false positives were reported. Experimental work indicated that ASFv may be detected in oral fluids prior to onset of clinical signs and even if only a small proportion of animals are infected. However, as the viral load, as measured by CT comparison, was much lower in oral fluids than individual tissue samples, the study indicated false negatives do occur (ASFv not detected in OF when blood/tissue samples were positive). This limits the potential use of swine OF as an official monitoring and surveillance tool in the event of an ASF outbreak in the U.S.

Therefore, the Swine Health Information Center is calling for proposals to develop methods to improve the detection of low levels of nucleic acid in OF through enhancements of sample pre-extraction treatment(s) or through improved extraction methodologies.

- The objective of the research is to identify sample treatments (for example centrifugation, nucleic acid concentration or other sample treatments) or extraction methods that improve the quantitative detection of PRRSV, IAV-S, and *Mycoplasma hyopneumoniae* nucleic acids in swine oral fluids.
- The research should include comparisons of the proposed sample treatments or extraction methods with the high throughput protocols currently in use in NAHLN veterinary diagnostic laboratories and result in treatments or extractions that are compatible with NAHLN high throughput testing.
- The proposal should include a clear description of the experimental design and statistical methods to be used in data analyses and provide for enough replicates and statistical power to be able to clearly show if the probability of detection x concentration differs among treatments or extraction methodologies for a particular PCR test.
- Since research has shown that it is easier to recover nucleic acids from intentionally spiked samples than from samples collected from infected pigs, the experimental design should include oral fluid samples collected from infected pigs. Proposals partnering with pork producers and their veterinarians to use oral fluid samples from naturally infected pigs to control costs will be prioritized.
- With the importance of improving swine oral fluid PCR sensitivity so it can be used with confidence as an official monitoring and surveillance tool, proposals with a timeline reflecting urgency will also be prioritized.