The monitoring of Salmonella in poultry has been in effect over the last few years, since most human cases of Salmonella have been associated with the consumption of eggs and poultry products. Poultry can become infected with different Salmonella serovars, frequently asymptptomatically. Some serovars, such as Pullorum and Gallinarum, are specific to birds, causing disease in these animals but rarely in humans, while others, such as Enteritidis, Typhimurium, and Heidelberg are able to infect a broad range of hosts.

Particularly, Salmonella Enteritidis can infect laying hens and contaminate eggs, which has been considered one of the main sources of human food-borne illness. Federal regulations require most egg producers to take steps to prevent the spread of Salmonella Enteritidis. At the ISU VDL, we are making every effort to assist you in complying with the requirements for Salmonella Enteritidis testing mandated by the FDA.

Salmonella rapid surveillance in poultry

According to the National Poultry Improvement Plan (NPIP), Salmonella monitoring has shifted from primarily bird culturing and serological testing to sampling various environmental sources. There are several challenges involved in detecting Salmonella from environmental samples, such as high microbial loads, which can lead to false negatives due to strong growth competitors and false positives due to closely related competitors, and low viability of Salmonella organisms present.

Sample type
According to the National Poultry Improvement Plan (NPIP), Salmonella monitoring has shifted from primarily bird culturing and serological testing to sampling various environmental sources. There are several challenges involved in detecting Salmonella from environmental samples, such as high microbial loads, which can lead to false negatives due to strong growth competitors and false positives due to closely related competitors, and low viability of Salmonella organisms present.

Sample process
Environmental swabs should be collected using pre-moistened swabs, placed into sterile bags and transported to the lab on cold packs. We can set up samples on the same day they arrive using their primary media with an added supplement, incubate for 16-22 hours, transfer to secondary enrichment, incubate for another 16-22 hours, place a strip test in each sample the following day and in 10 minutes have results (positive/negative). This method works really well to be able to get results to the client in 48 hours or less and allows the clients to move forward with their process sooner.

Salmonella identification
At the ISU VDL, we use a rapid, highly specific enrichment for Salmonella serogroup D1, which offers a better growth environment for Salmonella, helps control the growth of competing microorganisms, and provides Salmonella superior growth and recovery.

The media is quick and easy to prepare and there are no instrument costs or maintenance which allows for a cost-effective test for clients.
PCR clamping for selectively sequencing wild-type PRRSV in vaccinated herds

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**KEY POINTS:**

- Conventional ORF5 sequencing may not differentiate between wild-type or vaccine-like.
- Blocking the amplification of vaccine-like sequences is possible to increase the likelihood of wild-type amplification.
- Clamping allows the amplification of the wild-type with mixtures containing as little as 10% of a mixture with the vaccine-like.

Transmission of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is frequently monitored via open reading frame 5 (ORF5) sequencing. This data is used to identify new introductions of the virus as well as to determine whether the virus is wild-type or vaccine-like.

Since routine sequencing methods are often unable to differentiate wild-type from modified-live virus (MLV) PRRSV in vaccinated herds when both are present at the time of sampling, a tool which preferentially amplifies ORF5s of wild-type PRRSV in the presence of MLV vaccine virus would be highly beneficial.

“PCR clamping” technology has been described in which a modified oligonucleotide (“clamp”) is used to block PCR amplification of a specific sequence. Binding of the clamp to the DNA prevents PCR amplification and increases the likelihood of amplification of non-blocked sequences.

Here we describe a PCR clamping assay to suppress ORF5 amplification of Invega PRRS® MLV (I-MLV) vaccine to promote preferential amplification of wild-type PRRSV IF present also in the sample.

For clamp design, PRRSV sequences from GenBank or generated from clinical cases at ISU VDL were used to identify an “I-MLV” “signature sequence” present in sequences highly similar to I-MLV but not in wild-type PRRSV sequences. This oligonucleotide is included in the PCR reaction to block ORF5 amplification of I-MLV-like virus and enhances amplification of wild-type sequences in the sample. See Figure 1.

PRRSV PCR-positive samples previously characterized as “I-MLV vaccine-like” or “wild-type” based on ORF5 sequence were sequenced with and without the clamp. Weaker positive samples (CT >30) generally did not amplify regardless of whether or not the clamp was present. The remaining samples, all with ORF5 sequences 97 to 100% identical to I-MLV (N=30), resulted in a decrease or complete elimination of I-MLV ORF5 amplification. For the 22 samples <91% identity to I-MLV, there was a notable decrease in amplification with clamp compared to without clamp.

Previously characterized virus isolates representing MLV-like and wild-type PRRSV were mixed in ratios of 10:0 to 0:10, respectively, with and without clamp and subsequently sequenced. Generally, without the clamp added, the level of wild-type virus that was needed to reseed a wild-type sequence of high quality was a minimum of 70%. In contrast, when the clamp was added to the PCR reaction, high quality wild-type sequence could be obtained when the wild-type virus was present at as little as 10% of the mixture. See Figure 2 for sequencing data generated from a 50:50 mix of MLV and wild-type viruses.

The clamp has been used on several ISU VDL cases in which original sequencing results identified I-MLV-like virus and incorporation of the clamp also revealed the presence of a wild-type virus in the sample.

"Wild-type" PRRSV sequencing is available at ISU VDL to preference wild-type sequencing over I-MLV vaccine, and the same technology is currently in development for the Zoetis Fostera® PRRSV vaccine.