

Salmonella rapid surveillance in poultry



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.

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 asymptotically. 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.



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.

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.



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.



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Winter – 2019

NEWSLETTER



Sheila Norris Serology

Sheila grew up on a farm in Jackson County, Iowa. From a young age, she always enjoyed working with animals on the farm and felt that agriculture would be a part of her life. Thus, she attended Luther College in Decorah and transferred to Iowa State where she graduated with a Bachelor's degree in Dairy Science.

Sheila started working at the ISU VDL in 1991 as a lab technician and her role has evolved several times. She now serves as the lab manager for the Serology section. She works with a great group of people, and every day presents different challenges. Before working at the ISU VDL, she guided horseback rides in Rocky Mountain National Park. She has also worked for the USDA at the Plant Introduction Station and for a livestock auction and an engineering firm.

Sheila lives in Huxley with her husband Bill, and they have two children. Joshua graduated from Upper Iowa University with a degree in Forensic Science and Chemistry in 2018 and lives in Des Moines and works for Eurofins Scientific. Jessica is a junior at Hope College in Holland, MI where she majors in Dance and Business. In her free time, she enjoys spending time with her family, traveling and cooking.

STAFF HIGHLIGHT

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 ORF5 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 Ingelvac PRRS® MLV (I-MLV) vaccine to promote preferential amplification of wild-type PRRSV if also present 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 no discernable decrease in amplification with clamp compared to without clamp.

Previously characterized virus isolates representing I-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 generate 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 I-MLV and wild-type viruses.



Figure 1. Depiction of how the clamp prevents amplification (A) when a matching sequence is present in a sample, or has no effect (B) when a matching sequence is absent from the sample.

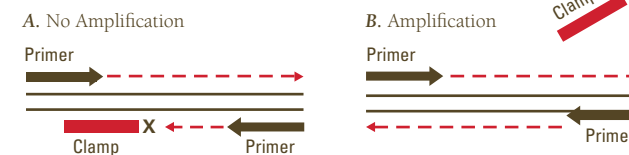
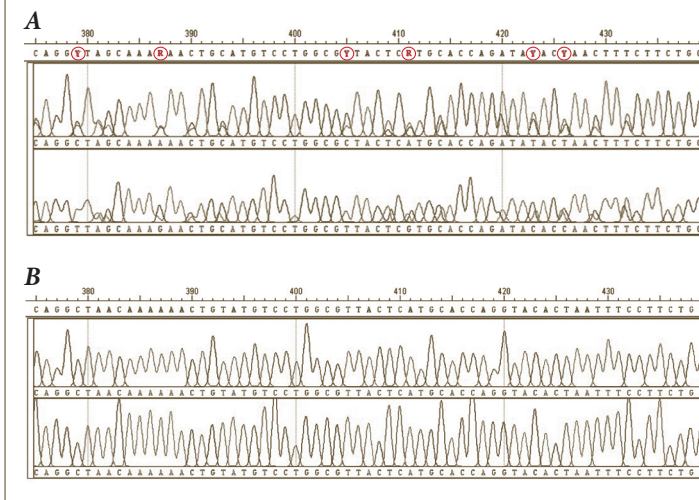


Figure 2. ORF5 sequencing data from a 50:50 mix of vaccine-like and wild-type PRRSV without (A) and with (B) clamp added to the PCR reaction. Note the ambiguous bases (double peaks with base designations circled in red) observed without clamp that are resolved when the clamp is included.



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 Foster® PRRSV vaccine.

ANNOUNCEMENTS:

Upcoming University Holidays:

New Year's Day – Wednesday, January 1st
Martin Luther King Day – Monday, January 20th

HATS will be closed on New Year's Day, but will be open for drop off cases on MLK Jr Day.



THANK YOU!

On behalf of everyone at the ISU VDL, thank you for your continued support. We hope everyone has a joyous holiday season and a Happy New Year!

Questions?

Please contact ISU VDL Client Services
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