Wisconsin Veterinary Diagnostic Laboratory
UNIVERSITY OF WISCONSIN–MADISON

Salmonella enterica subspecies *enterica* PCR and Culture Workflow at WVDL

Salmonella diagnostic testing can be challenging to interpret and apply to clinical or pathology samples. The WVDL has implemented changes to the workflow for the identification of *Salmonella enterica* subspecies *enterica.* These changes were based on existing data from published literature and data collected at the WVDL. We have analyzed the data collected in the past three years to provide clients with a better understanding of why the WVDL has designed its *Salmonella* testing scheme.

The WVDL uses real-time PCR to identify *Salmonella* nucleic acid and traditional culture methods to obtain live *Salmonella* isolates, which can be used for serotyping and susceptibility testing. The WVDL offers a *Salmonella* PCR that identifies the *Salmonella enterica* subspecies *enterica*. The *Salmonella* species PCR targets the *Salmonella* genus and the assay uses conserved genes common to all *Salmonella* species. The WVDL continues to culture the buffered peptone water (BPW) that was used for *Salmonella* PCR testing when the results are ≤ 35 C_T (cycle threshold).

Since 2015, the WVDL has been collecting data comparing our PCR and culture rates using the data collected from samples that obtain a 35 or lower C_T on the *Salmonella* species PCR. Table 1 demonstrates culture rates based on C_T value for 2020. At a C_T value of ≤25, the WVDL cultures *Salmonella*, of any serotype, 98.3% of the time. That rate drops as the C_T value increases demonstrating that less nucleic acid, specific to *Salmonella,* is present. At ≤35 C_T, we observe an 81.6% culture rate. It's important to note that when we pull out C_T values to those within a relatively high C_T value range that the culture rates drop. As an example, a C_T value between 25-30 produces a 91.6% culture rate by a C_T value between 30-35 is 61.7%.

Table 1: Culture rate for *Salmonella* species post multiplex PCR for 2020.

	≤25	**≤30**	**≤35**	**25-30**	**30-35**
Total Samples	118	308	500	191	193
***Salmonella* Isolated**	117	299	454	183	156
No *Salmonella* Isolated	1	9	46	8	37
Culture Rate	98.3%	94.2%	81.6%	91.6%	61.7%

For 2018, we found that 85.2% of samples with a ≤ 35 C_T value cultured *Salmonella* of any serotype (Table 2). Additionally, we found that a C_T value of ≤ 30 had a 94.9% culture rate. For 2017, we found that 80.7% of samples with a 35 or lower C_T value cultured *Salmonella* of any serotype (Table 3). Similar results were observed for the

years 2014 to 2016 (Table 4). Therefore, the WVDL culture rates post-BPW enrichment have remained relatively stable.

Table 2: Culture rate for *Salmonella* species post multiplex PCR for 2018.

***Salmonella* species PCR C_T Value**	**Culture Rate**
≤ 35	85.2%
≤ 30	94.9%
≤ 25	94.2%

Table 3: Culture rate for *Salmonella* species post multiplex PCR for 2017.

***Salmonella* species PCR C_T Value**	**Culture Rate**
≤ 35	80.7%
≤ 30	93.9%
≤ 25	97.6%

Table 4: Culture rate for *Salmonella* species post multiplex PCR for 2014-2016.

***Salmonella* species PCR C_T Value**	**Culture Rate**
≤ 35	77.8%
≤ 30	91.3%
≤ 25	97.9%

It's important to note that cultures rates do not take into account the culture of *Salmonella* from the same animal or sample, which could have resulted in a positive *Salmonella* culture, but a negative *Salmonella* culture from the BPW, which is used for the PCR. This data only examines the culture process of inoculating BPW with a sample, such as feces or intestine, and incubating for 18-20 hours. The BPW sample is split and an aliquot is used for PCR and a second aliquot is held for 24 hours until the PCR results are available and then BPW whose PCR obtains a C_T value of ≤35 is cultured in enrichment media and plated on selective plates. Therefore a different sample, from the same animal, may have been directly cultured using enrichment broths and selective plates and obtained a positive *Salmonella* culture when the BPW did not. The WVDL has observed that some *Salmonella* serotypes are enriched in BPW, an example is *Samonella* ser. Cerro, and others are difficult to culture from BPW, such as *Salmonella* ser. Dublin. Additionally, culture rate does not take into account animals that were treated with antibiotics prior to submission, which can result in a negative culture.

The WVDL has been observing an increase in the isolation of *Salmonella* ser. Cerro (see J Dairy Sci. Valenzuela et al. 2017), which has also been observed in New York and Pennsylvania. When a sample results in a high C_T value (low nucleic acid found in

the sample), which will not result in automatic culture, it is recommended that additional samples from a particular animal or farm with appropriate risk factors or history of salmonellosis should be taken and tested to prove salmonellosis.

The WVDL has implemented the following workflow to assist clients in obtaining the timeliest results. Diagnostic samples, which are enriched in BPW for ~18 hours and are run on the *Salmonella* species PCR are automatically submitted for bacterial culture when the PCR C_T value is ≤ 35 (culture rate of 81.6% in 2020). Clients who do not want this testing done should notify bacteriology or their case coordinator (608-262-5432). Clients who would like culture performed from a BPW sample that the C_T value was >35 should notify bacteriology or their case coordinator of that request.

The purpose of the continued culture on a low C_T value for the *Salmonella* species PCR positives is to obtain an isolate for further serotyping and possibly susceptibility. **If culture is successful, serotyping will automatically be performed, but will have an additional charge.** Clients who do not wish to have serotyping testing should notify the bacteriology section or their case coordinator. *Salmonella* serotyping can take multiple days to weeks if the isolate is typeable. Non-typeable isolates can be sent to NVSL if requested.

WVDL will no longer perform an antimicrobial susceptibility testing automatically for *Salmonella* isolates obtained from fecal samples. The WVDL will continue to do *Salmonella* serotyping automatically and will do antimicrobial susceptibility testing if it is requested by the submitting veterinarian. Some of the reasons for this change in laboratory policy are listed below:

1. There are no veterinary approved Clinical and Laboratory Standards Institute (CLSI) break-points for *Salmonella*. The only break-points available are for human use[1].
2. Oral antibiotics cause gut microbiome dysbiosis (less diversity) leading to an increased probability of *Salmonella* colonization and increased risk of invasive *Salmonella* complications[2].
3. Oral antibiotics increases the risk of antimicrobial resistance (AMR) for many different types of gut bacteria including *E. coli* and *Salmonella*[2].
4. Enteric *Salmonella* are often resistant to the most common classes of oral antibiotics that are approved for use in livestock.
5. Oral antibiotics increase the number of *Salmonella* superspreaders and their use also increases the duration of *Salmonella* shedding[3]. It has been estimated that superspreaders account for roughly 80% of *Salmonella* transmission[3]. Superspreaders are especially problematic since they cause an increase in environmental load of *Salmonella* which serves as a reservoir of new infections.
6. For dairy beef and veal operations, oral antibiotics particularly those that use

the aminoglycoside class of antimicrobial drugs increase the risk of drug residues being detected at the time of slaughter.
7. *Salmonella* are intrinsically resistant to the aminoglycoside class of antibiotics which includes Neomycin[4].
8. Some commercial non-medicated probiotics have good activity against enteric *Salmonella*, do not cause drug residues and have been shown to improve gut microbiome diversity[5,6]. This means there are viable alternatives to oral antibiotics in livestock that are relatively inexpensive.

Please be aware that when the WVDL cultures *Salmonella* serotypes from environmental samples, these isolates will not routinely undergo antimicrobial sensitivity testing.

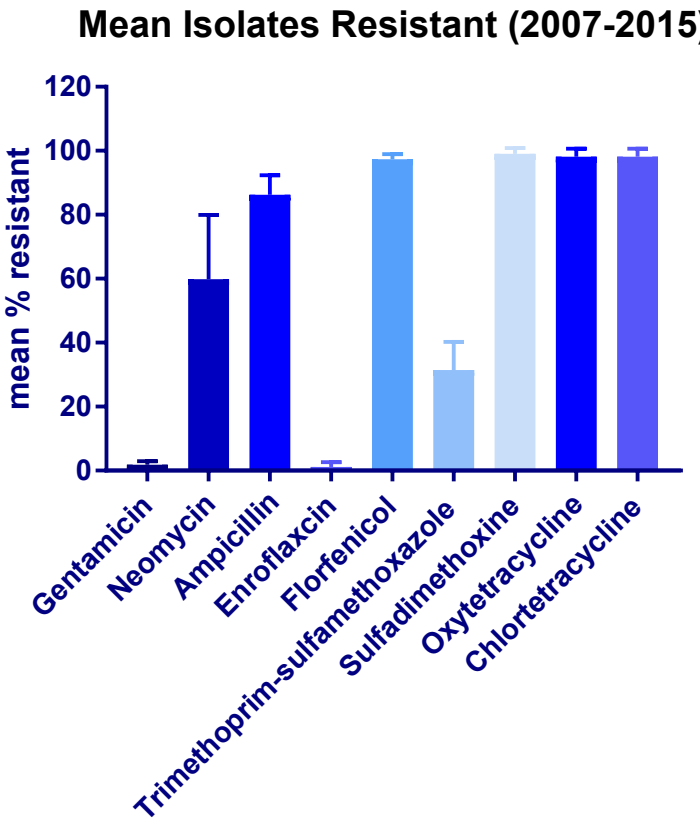
A sample is positive for *Salmonella* species PCR when the C_T < 40. A C_T value between 37 and 40 is considered a weak positive, where little nucleic acid is found and further sampling or testing may be necessary. This sample will not be automatically sent for culture. Clients can request culture on these samples by contacting bacteriology or their case coordinator.

Interpretation of PCR results requires that performance of the PCR assay is done with contamination control in mind at each step of extraction and amplification. At the WVDL, AAVLD approved PCR guidelines are followed to monitor and prevent environmental contamination. Proper sample acquisition requires practitioners to be cognizant of potential contamination from vaccines as well as other environmental sources such as clothing and gloves. *Salmonella* nucleic acid is typically prevalent in the environment on the modern dairy and poultry production facility. Use caution to not over interpret environmental samples submitted for PCR. Environmental samples should be cultured to confirm presence of viable pathogens.

Salmonella ser. Dublin does have a lower culture rate (~60%) and therefore it may be necessary to use the PCR results along with clinical history of the herd or animal tested to dictate changes to management practices or antimicrobial therapy. The antimicrobial sensitivity for *Salmonella* ser. Dublin has not changed in the recent past (Appendix 1). Data from WVDL shows that only enrofloxacin, gentamicin, and trimethoprim sulfa (TMS) have consistent susceptibility, but we remind the practitioner about the FDA restricted uses of fluoroquinolones and aminoglycosides in food producing animals. Fifteen of 27 isolates were susceptible to TMS.

In the United States, TMS only available for food animals in oral formulation, appropriate for use in calves <2-3 weeks of age in the pre-ruminant stage of life. Enrofloxacin is labeled for bovine respiratory disease complex pathogens and in dairy

animals less than 20 months of age only – off label use is strictly prohibited. Aminoglycosides are still part of a voluntary ban and carry 18-24 month slaughter withhold.



Appendix 1

These data indicate that enrofloxacin, trimethoprim sulfamethoxazole (TMS), and gentamicin should be considered potential therapeutic antimicrobials if necessary. Enrofloxacin is labeled for respiratory disease and in dairy animals less than 20 months of age only- off label use is illegal. TMS is only available in oral formulations, appropriate for use in calves < 2-3 weeks of age. Aminoglycosides are still part of a voluntary ban and carry a 18-24 month slaughter withhold.

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