Wisconsin Veterinary Diagnostic Laboratory
UNIVERSITY OF WISCONSIN–MADISON

Salmonella 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* that utilize existing data from published literature and data collected at the WVDL to guide these changes. We have analyzed the data collected in the past 1.5 years to provide clients with a better understanding into why the WVDL has designed its *Salmonella* testing scheme.

Background:

The WVDL uses 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 multiplex *Salmonella* PCR that identifies the *Salmonella enterica* subspecies *enterica* and *Salmonella enterica* subspecies *enterica* serotype (or serovar) Dublin, which is endemic in the cattle population. The *Salmonella* species PCR targets the entire *Salmonella* genus and the assay uses conserved genes common to all *Salmonella* species. *Salmonella enterica* ser. Dublin PCR is specific to only *S. enterica* ser. Dublin. Fecal, intestinal, and tissue samples are enriched in buffered peptone water (BPW) for more than 18 hours prior to PCR. Samples that yield a cycle threshold (C_T) value less than 40 will be considered a positive. Although, the PCR may yield a positive result, a positive culture result from this same sample may be difficult to obtain. The table below shows the culture rate as compared to the C_T value obtained on PCR.

***Salmonella* species PCR C_T Value**	**Number of Tests Examined**	**Culture Rate**
≤ 35	746	82%
≤ 30	462	92%
≤ 25	202	95%

Note: culture rate does not take into account animals that were treated with antibiotics prior to submission.

Culture rates for *Salmonella* ser. Dublin are variable depending on the sample type. Literature suggests low culture rates for *Salmonella* ser. Dublin from feces of which the WVDL can confirm. However, the WVDL has a higher culture success rate from tissues. A collection of these data are represented in the chart below.

Wisconsin Veterinary Diagnostic Laboratory
UNIVERSITY OF WISCONSIN–MADISON

***Salmonella* ser. Dublin PCR C_T Value**	**Number of Tests Examined**	**Culture Rate for *Salmonella* ser. Dublin (Group D1)**		**Culture Rate for *Salmonella* ser. Cerro (Group K)**
≤ 35	162	60%	17%	9%
≤ 30	91	84%	4%	2%
≤ 25	54	91%	4%	2%

Note: culture rate does not take into account animals that were treated with antibiotics prior to submission.

The WVDL has observed an increase in the isolation of *Salmonella* ser. Cerro. Additionally, we have observed the co-culture of *Salmonella* ser. Dublin and other serovars such as *Salmonella* ser. Cerro, which is demonstrated in the table above. Therefore, we continue to culture *Salmonella* species PCR ≤ 35 even if the *Salmonella* ser. Dublin PCR is also positive even at a high C_T value as other *Salmonella* serovars may also be present. When a sample results in a high C_T value, 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 or obtain antimicrobial sensitivity patterns.

Testing Workflow:

The WVDL has implemented the following workflow to assist clients in obtaining the timeliest results. Diagnostic samples, which are enriched in BPW and are run for the *Salmonella* PCR panel, are automatically submitted for bacterial culture when the PCR C_T value is ≤ 35 for the *Salmonella* spp. PCR (culture rate of 82%). 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 C_T value was >35 should notify bacteriology or their case coordinator. Bacterial culture is initiated for samples that result in a C_T value of ≤ 35 for the *Salmonella* spp. PCR regardless of *S. enterica* ser. Dublin PCR C_T value, which is due to the lower culture success rate for *Salmonella* ser. Dublin and the antimicrobial sensitivity for has not changed in the recent past. The purpose of the continued culture on a low C_T value for the *Salmonella* spp. with a *Salmonella* ser. Dublin PCR positive is to check for multiple serovars as demonstrated in the table above. If culture is successful, serotyping and sensitivity testing will automatically be performed at an additional charge. Clients who do not wish to have serotyping and/or culture should notify the bacteriology section or their case coordinator. *Salmonella* serotyping can take multiple days to 3 weeks if the isolate is typeable. Non-typeable isolates can be sent to NVSL if requested. *Salmonella* spp. cultured from environmental samples will not routinely undergo antimicrobial sensitivity testing.

Wisconsin Veterinary Diagnostic Laboratory
UNIVERSITY OF WISCONSIN–MADISON

The WVDL recommends the following tests depending on the age of the bovine animal:
Calves < 4 months: *Salmonella* PCR Panel; a C_T value is ≤ 35 for the *Salmonella* spp. PCR will automatically trigger bacterial culture. The PCR is suggested instead of bacterial culture because *Salmonella* ser. Dublin is difficult to culture.
Adult animals and calves > 4 months: *Salmonella* culture or *Salmonella* PCR Panel; a C_T value is ≤ 35 for the *Salmonella* spp. PCR will automatically trigger bacterial culture. If salmonellosis is suspected, requesting culture immediately will save one day as compared to bacterial culture after PCR, but a loss of sensitivity might occur.

Interpretation:
A sample is positive for *Salmonella* spp. or *Salmonella* ser. Dublin PCR when the $C_T < 40$. A C_T value between 36 and 40 is considered a weak positive, where little nucleic acid is found and further sampling or testing may be necessary.

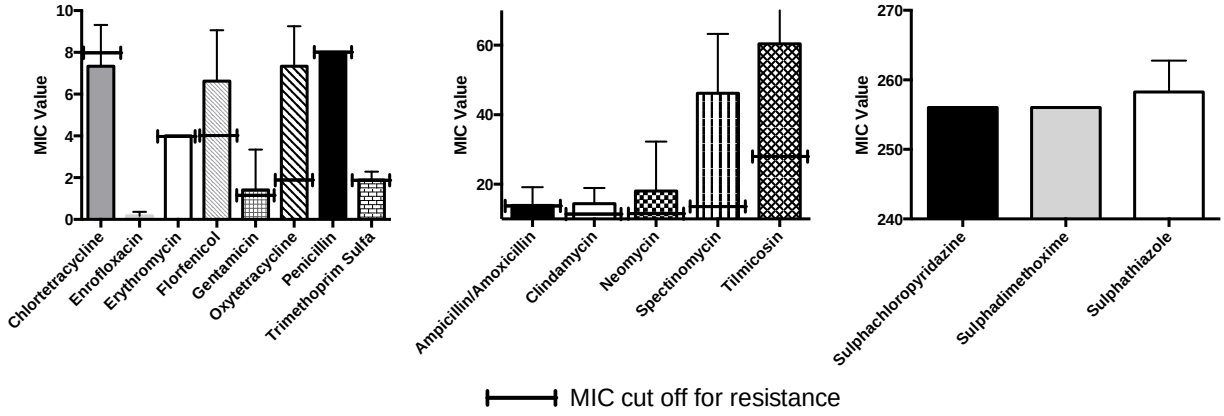
Interpretation of PCR results requires that performance of the PCR assay is done with contamination control in mind at each step of extraction and amplification. At the WVDL, AAVLD approved PCR guidelines are followed to monitor and prevent environmental contamination. Proper sample acquisition requires 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.

The *Salmonella* PCR is multiplexed and both reactions are running in the same well at the same time. These two reactions have slightly different sensitivities. By design, the *S. enterica* ser. Dublin reaction is more sensitive. Therefore, it is possible that a reaction could be *S. enterica* ser. Dublin positive and *Salmonella* species negative. WVDL data shows that the above scenario occurs in ≤ 5% of all *Salmonella* PCR reactions when *S. enterica* ser. Dublin is also positive. The *Salmonella enterica* ser. Dublin primers detect *S. enterica* ser. *gallinarium* and *S. enterica* ser. *pullorum in silico*. The *Salmonella enterica* ser. Dublin PCR should not be requested on poultry cases.

Salmonella ser. Dublin positive PCR samples with *Salmonella* spp. PCR $C_T \geq 35$ are not routinely cultured because *Salmonella* ser. Dublin can be quite challenging to grow and the antimicrobial sensitivity for the host adapted strain has not changed in the recent past. (Appendix 1). Data from WVDL shows that only enrofloxacin, gentamicin and trimethoprim sulfa (TMS) should be considered as potential therapeutic antimicrobials. 15 of 27 isolates were susceptible to TMS.

Trimethoprim sulfa is only available in oral formulation, appropriate for use in calves <2-3 weeks of age. Enrofloxacin is labeled for respiratory disease and in dairy animals less than 20 months of age only – off label use is illegal. Aminoglycosides are still part of a voluntary ban and carry 18-24 month slaughter withhold.

Appendix 1



MIC values for WVDL *Salmonella* Dublin Isolates (2007-2015)

Limited dataset includes isolates from each year (27 total isolates). Bars represent mean and error bars are SD from mean. This data shows that only enrofloxacin, gentamicin, and trimethoprim sulfa (TMS) should be considered potential therapeutic antimicrobials. 15 of 27 isolates were susceptible to TMS. Trimethoprim sulfa is only available in oral formulation, appropriate for use in calves <2-3 weeks of age. Enrofloxacin is labeled for respiratory disease and in dairy animals less than 20 months of age only – off label use is illegal. Aminoglycosides are still part of a voluntary ban and carry 18-24 month slaughter withhold.