Wisconsin Veterinary Diagnostic Laboratory
UNIVERSITY OF WISCONSIN-MADISON

Salmonella PCR and Bacteriology Testing 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.

Background:

The WVDL uses PCR to identify *Salmonella* nucleic acid and traditional culture methods to obtain live *Salmonella* isolates, which can be used for 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 refers to the entire *Salmonella* genus and the assay uses conserved genes common to all *Salmonella* species. *Salmonella enterica* ser. Dublin PCR refers 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 36 will be considered positive. Although, the PCR may yield a positive result, a positive culture result from this same sample may be difficult to obtain. Internal data has demonstrated that C_T values of ≤ 35 for the *Salmonella* species have a higher success rate of retrieving a *Salmonella* isolate than C_T values greater than 35. However, for the *Salmonella* ser. Dublin PCR, culture rates are much less successful in which a C_T of ≤ 32 for *Salmonella enterica* ser. Dublin PCR has a 60% culture rate. 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. Clients who do not want this testing done should notify bacteriology (608-262-5432 ext. 2115). Clients who would like culture performed from a BPW sample that C_T value was >35 should notify bacteriology. 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. The purpose of the continued culture on a low C_T value for the *Salmonella* spp. with a *S. enterica* ser. Dublin PCR positive is to check for multiple serovars. If culture is successful,

serotyping and sensitivity testing will automatically be performed at an additional charge. *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.

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.

Interpretation:

A sample is positive for *Salmonella* spp. or *Salmonella* ser. Dublin PCR when the $C_T \geq 36$. 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.

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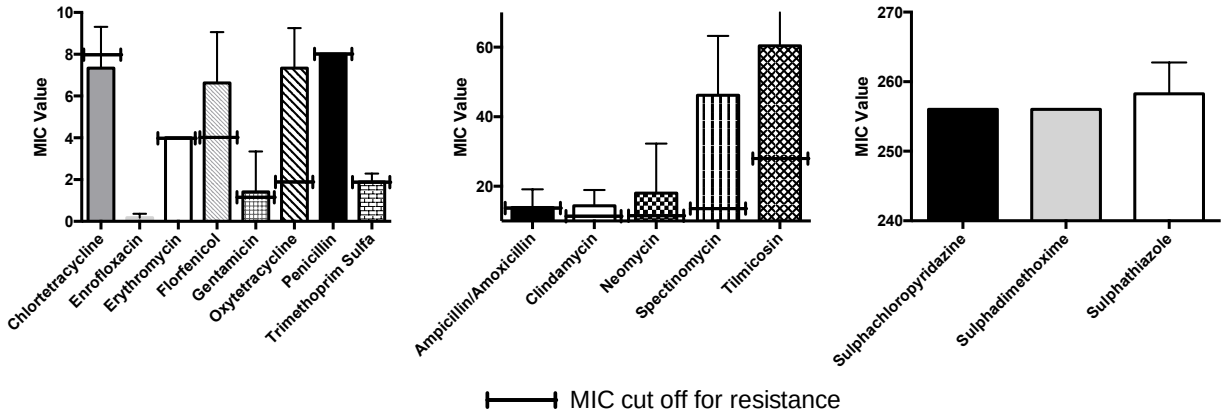
Salmonella ser. Dublin positive PCR samples with *Salmonella* spp. PCR $C_T \geq 35$ are not routinely cultured because *S. enterica* ser. Dublin can be quite challenging to grow and the antimicrobial sensitivity for the host adapted strain has not changed in the recent past (Appendix 1). Data from WVDL 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.