Wisconsin Veterinary Diagnostic Laboratory
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Salmonella 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, as of July 16, 2017, 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 three years to provide clients with a better understanding into 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 multiplex *Salmonella* PCR that identifies the *Salmonella enterica* subspecies *enterica* and the gene target vagC, formerly specific to *Salmonella* serotype/serovars Dublin. The *Salmonella* species PCR targets the entire *Salmonella* genus and the assay uses conserved genes common to all *Salmonella* species.

Upon recent examination of the gene target for the *Salmonella* ser. Dublin PCR, the WVDL discovered that this gene can now be harbored in *E. coli, Enterobacter,* and *Citrobacter*. At this time, the WVDL does not know the exact prevalence of this gene in non-*Salmonella* ser. Dublin bacteria. The WVDL has suggestive evidence that the prevalence of this gene, in our submitting population, is low (3-6% of tests on fecal and tissues samples per year).

Because *Salmonella* ser. Dublin is endemic in the cattle population and diagnosis on a farm has a high consequence, the WVDL will continue to offer the *Salmonella* ser. Dublin PCR, now called VagC PCR, as it still has significant clinical utility. We will continue to develop a new PCR assay for a gene target that is specific for *Salmonella* ser. Dublin.

However, effective 7/16/2018, the WVDL has changed the name of the *Salmonella* ser. Dublin PCR to the VagC PCR. VagC is the gene target for the *Salmonella* ser. Dublin PCR that is no longer specific to *Salmonella* ser. Dublin. As stated above, the WVDL has suggestive evidence that 94-97% of VagC PCR positives are specific to *Salmonella* ser. Dublin. This PCR is run as a multiplex with the *Salmonella* species PCR and results will be reported for both the *Salmonella* species and VagC.

- **Negative VagC results are reported as negative**, as this cross reaction does not affect negative results. This result can be used as a negative for *Salmonella* ser. Dublin.
- **Positive VagC PCR results that do not have a positive *Salmonella* species PCR results will be reported as positive, but should be interpreted as a possible cross-reaction with a non-*Salmonella* ser. Dublin bacterium.**
    - C_T values <35 will be automatically set up for culture and sensitivity, as is our current practice (see below for further information)
    - Correlate this result to clinical history on the farm and for the animal sampled
    - Consider confirmatory testing with ELISA
    - Consider testing additional animals – necropsy and culture of tissues is the best sample to recover *Salmonella* ser. Dublin
- **Positive VagC PCR results along with positive *Salmonella* species and VagC gene target should be interpreted as positive in conjunction with the clinical history of the herd and of the animal tested.**
    - C_T values <35 will be automatically set up for culture and sensitivity, as is our current practice (see below for further information)
- We do not recommend making management decisions for a herd or an animal on a single PCR test result. Clinical signs of the animal tested and history of the herd are very important factors to be considered. Further testing may be in the best interest of the herd owner.

Since 2015, the WVDL has been collecting data comparing our PCR and culture. Prior to 7/16/18, the WVDL automatically cultures all samples that obtain a 35 or lower C_T (cycle threshold) on the *Salmonella* species PCR. For 2017, we found that 80.7% of samples with a 35 or lower C_T value cultured *Salmonella* of any serotype (Table 1). Interestingly, we also observed that if both the *Salmonella* species PCR and the *Salmonella* ser. Dublin (as of 7/16/18 called by the target name, VagC) PCR are less than or equal to 35, we cultured *Salmonella* ser. Dublin 57.3% (Table 2). Therefore as of 7/16/18, we have been automatically culturing samples that obtain a 35 or less C_T value for VagC PCR, as our recovery rate is remarkably high. Previous reports demonstrate much lower culture rates for *Salmonella* ser Dublin (0-25%; Nielsen et al., 2013). We believe this to be best practice to confirm the VagC PCR result by using culture when there is a high likelihood of recovery. The tables below demonstrate our culture rates for *Salmonella* post *Salmonella* species and *Salmonella* ser. Dublin PCRs.

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Table 1: Culture rate for *Salmonella* species post multiplex PCR for 2017.

***Salmonella* species PCR C_T Value**	**Number of Tests Examined**	**Culture Rate**
≤ 35	693	80.7%
≤ 30	479	93.9%
≤ 25	164	97.6%

Table 2: Culture rate for *Salmonella* ser. Dublin post multiplex PCR for 2017.

***Salmonella* ser. Dublin PCR C_T Value**	**Number of Tests Examined**	**Culture Rate for *Salmonella* ser. Dublin (Group D1)**
≤ 35	118	57.3%
≤ 30	94	76.0%
≤ 25	54	95.0%

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

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, which will not results 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 or obtain antimicrobial sensitivity patterns.

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 for the *Salmonella* PCR panel (*Salmonella* species and VagC PCR), are automatically submitted for bacterial culture when the PCR C_T value is ≤ 35 for the *Salmonella* species or VagC PCR (culture rate of 80.7%). 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 of that request.

The purpose of the continued culture on a low C_T value for the *Salmonella* spp. or VagC PCR positives is to obtain an isolate for further serotyping and susceptibility. 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* species cultured from environmental samples will not

routinely undergo antimicrobial sensitivity testing.

A sample is positive for *Salmonella* species or VagC 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.

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 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. 15 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.

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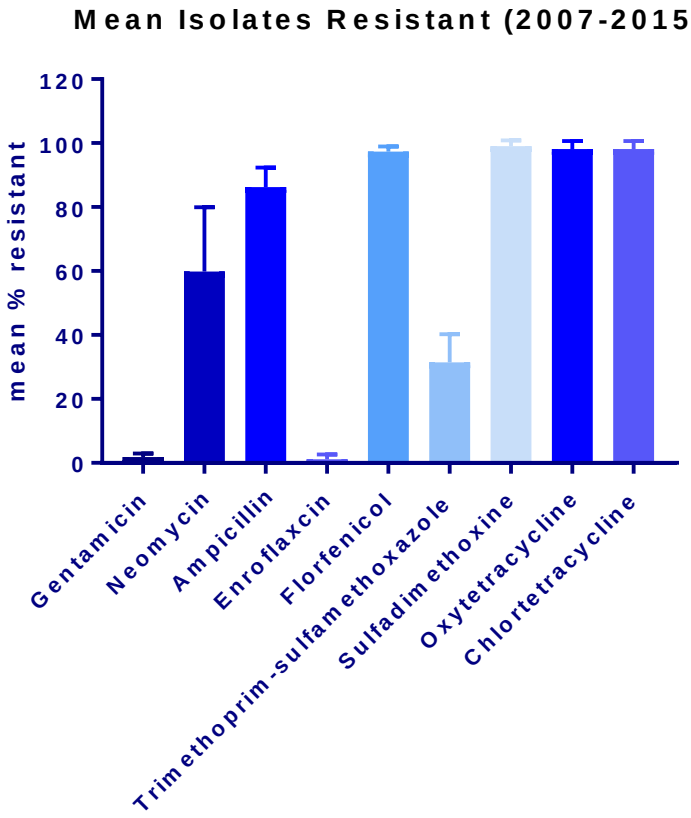
Appendix 1



These data indicate that enrofloxacin, trimethoprim sulfamethoxazole (TMS), and gentamicin should be considered potential therapeutic antimicrobials. 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.