Wisconsin Veterinary Diagnostic Laboratory
UNIVERSITY OF WISCONSIN–MADISON

PCR Assay Diagnostic Aid

Interpretation of WVDL Real Time PCR assays

In a real time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to exceed background levels and cross a pre-set threshold. Ct values are inversely proportional to the amount of target nucleic acid detected in the sample (i.e., the lower the Ct value the greater the amount of target nucleic acid detected in the sample). WVDL real time assays typically undergo 40 cycles of amplification.

In general, a positive PCR result only indicates that the pathogen's genetic material is detected in that sample and the genetic material could be from live and/or dead organisms. We always recommend performing other testing (such as antibody detection or bacterial/viral culture) in conjunction (parallel) with PCR or as follow-up testing (serially) to ensure clients have a complete profile of the health of the animal in order to make an accurate diagnostic evaluation.

Interpretation of WVDL PCR assays (including MAP PCR):

Ct Value	**Interpretation**	**Explanation**
Ct value ≤ 37.0	Detected	Reaction indicative of the presence of target nucleic acid. The lower the Ct value, the higher the amounts of nucleic acid present in the sample.
Ct value 37.1-40.0	Inconclusive	Weak reaction which could represent early or late infection, residual vaccine or environmental contamination. Follow up testing is recommended.
Ct value 0.0	Not Detected	No detection of the target nucleic acid.
No Ct value	Indeterminate	This results is specific for **MAP PCR** when reaction was observed from only one of the 3 PCR targets or the IS900 target did not show a reaction. This can be indicative of other Mycobacterium or nonspecific reactivity. A follow up MAP PCR or fecal culture is recommended.

BVD PCR Interpretation and Recommendations

When screening for persistent infection (PI) of BVDV, the Ct value of BVDV individual PCR reactions can be used as a general guideline to assess the PI status of an animal. Based on the data that WVDL has generated, those animals with Ct values of approximately 31 or less may be persistently infected. These animals should be retested in 4 weeks, animal with acute infection should have clear the virus in their system, while the PI animal should remained infected, the follow up testing is needed to more accurately determine their true status.

Escherichia coli Virulence Factor Real Time PCR Panel

WVDL performs real time PCR for the presence of 6 toxins/virulence factors on *E. coli* bacterial isolates as well as samples submitted for diagnostic testing. The significance of the detected genes should be interpreted with the patient clinical picture and/or histopathological findings of known historical risk factors in mind.

- **STa:** encodes for heat stable enterotoxin. This is a plasmid encoded protein that is a potent stimulant of intestinal chloride and bicarbonate secretion, resulting in fluid accumulation in the intestines and blockage of absorption of NaCl and water from the apical tip cells.
- **Stx1:** encodes for Shiga-like toxin (verotoxin) 1 [SLT-I] which is most likely identical to the toxin produced by Shigella spp. Shiga-like toxin 1 is neutralized by antibody specific for shiga toxin.
- **Stx2:** encodes for Shiga-like toxin (verotoxin) 2 [SLT-II] which is most likely a variant to the toxin produced by Shigella spp. Shiga-like toxin 2 is not neutralized by antibody specific for shiga toxin.
- **eaeA**: encodes for Intimin, which allows for intestinal cell attachment in Enteropathogenic *E. Coli* (EPEC) strains.
- **K99:** encodes for the mannose resistant adhesin K99 which allows attachment to glycoproteins on the surface of epithelial cells of the jejunum and ileum. Receptors on the epithelial cells only exist transiently during the first week or so of life in calves and lambs.
- **F41:** encodes for fibrial adhesion first recognized as a second mannose-resistant hemagglutinin produced by Enterotoxigenic E. *coli* (ETEC) strains. This has been shown to be pathogenic in cattle and swine.

Clostridium perfrigens Toxinotyping

WVDL offers a *Clostridium perfrigens* toxinotyping (A, B, C, D, E, or F) PCR assay for *C. prefringens* isolates by identifying 6 toxin genes (alpha (cpa), beta (cpb), epsilon (etx), iota (iA), enterotoxin (cpe) and beta2 toxin (cpb2). This is a presence/absence PCR and does not have a corresponding Ct value.
Toxin types are determined based on the chart below.

	Major toxins				Minor toxins	
Toxinotype	**cpa**	**cpb**	**etx**	**iA**	**cpe**	**cpb_2**
A	+	-	-	-	-	+/-
B	+	+	+	-	+/-	+/-
C	+	+	-	-	+/-	+/-
D	+	-	+	-	+/-	+/-
E	+	-	-	+	+/-	+/-
F	+	-	-	-	+	+/-