



## TECHNICAL REPORT

### ADVANTAGES OF THE AQUILA ACCUTASVET PLATFORM FOR MOLECULAR DIAGNOSTICS IN THE POULTRY INDUSTRY

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## BACKGROUND

### *Aquila Hydrogel Platform*

Aquila Diagnostic Systems (Aquila) has developed a unique real-time polymerase chain reaction (qPCR) platform that allows for point-of-care diagnostic testing without the need for highly skilled personnel. The AccutasVET DNA test platform is based on our patented hydrogel technology which is designed to contain all reagents necessary for the identification of specific pathogens. The hydrogels have a long shelf-life and allow detection from minimally processed samples. The platform allows for direct sampling from raw samples (e.g. feces) with minimal sample processing which results in more accurate detection of targets, which include bacteria, viruses, and parasites. The hydrogel is preloaded in small tubes to which processed sample is added. The tubes are then loaded into a small footprint benchtop thermocycler instrument which is run in the field or close to the barn. Results are available in approximately 1.5 hours.

### *Aquila Poultry Surveillance Product*

The Aquila technology has been adapted and tested for a variety of poultry targets including:

1. *Eimeria spp.*
2. *Clostridium perfringens*
3. *Salmonella spp.*, and
4. *Cambylobacter jejuni*.

These pathogens increase poultry mortality and morbidity and cause substantial economic loss. The current protocol for testing for these pathogens involves sending dead birds to a pathology lab for post mortem diagnostics, which can take 24-48 hours or more. During this time, the disease burden and economic losses to the producer can increase rapidly. Aquila's AccutasVET DNA tests have a simplified workflow that allows veterinarians to quickly monitor for these pathogens as results are available within 2 hours, which leads to rapid treatment and productivity gains.

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## FIELD TRIAL

In mid 2017 Aquila transported 2 AccutasVET qPCR Instruments, AccutasVET DNA Test Devices, and all required sample processing equipment (centrifuge and heat block) to the CPC facility in Abbotsford, BC. Equipment unpacking and set-up took less than 15 min. CPC technical staff and veterinarians received an on-site orientation to the equipment and the product instructions for use. All sample collection, processing and testing for the field trial was performed by CPC staff. Appropriate samples (fecal droppings, cecal droppings, and environmental swabs) were collected by CPC and split for testing using (A) AccutasVET DNA assays, and (B) the reference test method at an accredited laboratory.



Bench top instrument



Tubes of hydrogel

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## FIELD TRIAL RESULTS

### 1) *EIMERIA SP*

Seven species of the genus *Eimeria* cause coccidiosis in poultry, leading to intestinal lesions, diarrhea, and mortality. Moreover, coccidiosis may serve as a predisposing factor for further infection by *Escherichia coli* (*E. coli*) and *Clostridium*. The AccutasVET *Eimeria* assay contains primers for a consensus region of the *Eimeria* 5S rRNA gene, which allows detection of *Eimeria* DNA from poultry fecal.

Eighteen poultry fecal droppings were collected by CPC from four different flocks (LWU, LWD, BWU, & BWD) weekly, and split for testing using (A) AccutasVET *Eimeria* DNA Test, and (B) Reference testing using an OPG (oocyst per gram) assay at the University of Guelph Animal Health Laboratory. Fresh samples were sent for the reference testing, while AccutasVET DNA tests were performed on fecal material that had been stored frozen at -20 °C for up to 11 weeks.

- The AccutasVET *Eimeria* test showed 100% sensitivity compared to the reference test.

- AccutasVET also identified two reference negative samples as positive (“false positive”), leading to 50 % specificity. The discrepancy may result from the sensitive nature of qPCR test, as well as from detecting DNA from non-oocyst *Eimeria* DNA.

## 2) *CLOSTRIDIUM PERFRINGENS*

Excessive intestinal growth of two types of toxin-producing *Clostridium perfringens* (*C. perfringens*) cause gut lesions in poultry. The acute form of the disease increases flock mortality. To identify both types of *C. perfringens* (i.e., Type A and Type B), the AccutasVET *C. perfringens* DNA test uses primers targeting the  $\alpha$ -toxin gene.

Eighteen poultry fecal samples were tested on the AccutasVET system by CPC staff at their production facility. Split samples were sent to BC Animal Health Centre for reference testing by anaerobic culture for *Clostridium*. Fresh samples were sent for the reference testing, while AccutasVET DNA tests were performed on fecal material that had been stored frozen at -20 °C for up to 11 weeks. The AccutasVET *C. perfringens* test shows 100% sensitivity compared to the reference test.

- It identified four reference negative samples as positive (“false positive”), leading to 33% specificity. The discrepancy may result from detecting DNA from non-viable *C. perfringens*.

## 3) *SALMONELLA*

The intestinal tract of poultry is most commonly colonized by *Salmonella typhimurium* and *Salmonella enteritidis*, which cause paratyphoid infection. Moreover, *Salmonella* in poultry and poultry products is a significant source of foodborne disease in humans. Consensus primers targeting the *Salmonella* invasion gene *invA* to detect all subtypes of *Salmonella* were designed and validated in collaboration with Vaccine and Infectious Disease Organization (VIDO), SK. The assay has been optimized for testing a number of different sample matrices including feces, fecal swabs, cloacal swabs, and environmental swabs.

Eight fresh environmental swabs were collected by CPC from four different poultry barns. The samples were split for on-site testing using the AccutasVET *Salmonella* DNA test, and reference testing by culture-based method at the Animal Health Center in Abbotsford, BC. Only 2 samples were positive in reference testing but all samples came up positive in AccutasVET test.

- One of the reasons behind this discrepancy is that while culture based reference method only detects viable organisms, AccutasVET tests for presence of DNA without discriminating dead and viable organisms.

- It will be necessary to incorporate viability discrimination capability into AccutasVET Salmonella Test for its successful field application in future.

#### 4) **CAMPYLOBACTER JEJUNI**

Undercooked poultry meat contaminated with *Campylobacter jejuni* (*C. jejuni*) is a significant cause of campylobacteriosis (an enterocolitis) in humans. It is the leading bacterial cause of sporadic enteritis in developed countries. Primers targeting the *hipO* gene for specific detection of *C. jejuni* were designed at VIDO. Primer specificity was evaluated by testing on 4 homologous strains (*Campylobacter* strains CJ8, 69, 97 and 304) and 7 heterologous strains (*E. coli* strains 81 and 94, *Enterobacter cloaca*, *Proteus mirabilis*, *Citrobacter freundii*, *E. coli*, and *S. Enteritidis*). AccutasVET *C. jejuni* DNA Test was optimized for seamless use of a number of sample matrices, including feces, cloacal swabs, cecal droppings, and cecal pouch contents.

Sixteen fresh cecal droppings were collected by CPC from four different poultry barns. The samples were split for testing using the AccutasVET *C. jejuni* DNA test, and the reference method. Testing on the AccutasVET system occurred on-site, while reference testing was performed using a culture-based assay at the Animal Health Center in Abbotsford, BC.

- No positive samples were identified by either the reference method or the AccutasVET test. More samples need to be tested to detect positives and to determine assay sensitivity.
- Specificity of the AccutasVET was 100%.

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## SUMMARY

- The AccutasVET DNA tests are sensitive, specific, and allow for multi-parameter testing of important poultry pathogens. It frequently out performs the reference test and will detect more positive samples
- The Aquila AccutasVET system is extremely easy to use. It can be used in a laboratory or field setting, and technicians can be trained to use the system on-site.

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## NOTE

A longer version of this report with more technical details is available in the Aquila Poultry White Paper. A copy can be made available by contacting Aquila media@aquiladiagnostics.com