Current Advances

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Gallant Custom Laboratories Inc.

Providing Unique Technology for Animal Health

***Escherichia coli* Virotyping**
Replacing Serotyping with PCR Testing

Change in *Escherichia coli* testing at Gallant

Gallant is moving away from serotyping *Escherichia coli* (*E. coli)* strains and is replacing it with a more precise method using PCR technology that detects genes for specific virulence factors associated with pathogenic *E.coli*. The new PCR test will enable Gallant Labs to better identify and characterize pathogenic *E. coli* strains that cause disease in animal herds or poultry flocks. The information produced from PCR testing will be useful in understanding the involvement of pathogenic *E. coli* and the selection of strains for inclusion in a targeted bacterin.

Reason for Change:

Serotyping is a laboratory technique that employs antisera directed at cell wall, capsular or adhesion antigens on the surface of *E. coli* cells to differentiate and categorize *E. coli* isolates. Serotyping has historically been an effective tool but has become less discerning with increased incidences of cross-reactive and untypeable *E. coli* strains that confound diagnostic information. Furthermore, the production of the antisera used in serotyping is being discontinued, making it unavailable to Gallant. Consequently, Gallant has decided to shift to PCR-based testing methods for *E. coli* detection and characterization. PCR is a sensitive and specific scientific tool commonly used for the detection and identification of an array of animal pathogenic organisms. In addition, the actual genes carried by microorganisms that are associated with pathogenicity can be identified.

The new *E. coli* PCR Test:

PCR testing methods developed to specifically detect, characterize and categorize pathogenic *E. coli* were shared with Gallant by Dr. John Fairbrother of the *Escherichia coli* Laboratory (EcL), University of Montreal. EcL is an OIE Reference Laboratory for *E. coli* and it is their mandate to validate, train and share *E. coli* testing methods. Complete information about EcL, the OIE mandate and *E. coli* testing methods are available at www.apzec.ca.

E. coli PCR testing at Gallant was validated using known reference strains provided by EcL and is based on a set of 13 virulence factors that include the genes for *E. coli* toxins LT, STa, STb, Stx1, Stx2, CNF, Aerobactin and Tsh and *E. coli* fimbriae/adhesion genes F4, F5, F18, P and Eae. Depending on the virulence factors detected, the *E. coli* culture(s) tested can be pathotyped (Table 1) as ETEC (Enterotoxigenic *E. coli*), EPEC (Enteropathogenic *E. coli*), STEC (Shiga toxin producing *E. coli*) or ExPEC (Extraintestinal pathogenic *E. coli*).

Gallant's *E. coli* PCR Testing Procedure:

Much of our research and development focused on the optimum screening and isolation procedure to pin-point virulent *E. coli* strains. We concluded that the best and most cost-effective initial screen was to pool up to 3 samples, followed by an overnight enrichment step and PCR testing. At the time of pooling, the samples are plated onto MaConkey (MAC) and Blood agar (BA) plates to determine the presence and predominance of any *E. coli*. Both of these analyses provide critical information. Plating gives a visual impression of the organisms in the sample, and the PCR test detects if virulence factor(s) of **pathogenic *E. coli*** are present or not.
If any virulence factors **are** detected, secondary PCR testing is performed on **individual** *E. coli* isolates retrieved from the original MAC and/or BA plates to determine if they contain any of the virulence factors identified in the original screen. Positive isolates are then evaluated for antimicrobial susceptibility and stored for future use. The diagnostic report issued will list the susceptibility results and virotype of each unique isolate.

***Escherichia coli* classification**		
Pathotype	***Associated Toxic Genes***	***Associated Adhesion Genes***
ETEC	LT, STa, STb	F5 (bovine) F4 and F18 (swine)
EPEC	EAE	
STEC	STx1, STx2	EAE
ExPEC	Aerobactin, Tsh, CNF	P

Table 1– Classifying *E. coli* into Pathotypes based on Virulence Factors Detected

Image 1– Gallant's Diagnostic Laboratory: Continues to offer remarkable services to meet the clients' needs.

A primary PCR of pooled samples can be used to screen a large number of samples for *E. coli* virulence factors, and results are available quickly. For negative results, there is no need for further testing – reducing the cost for the producer. For positive results, the veterinarian can decide to end testing after the initial screen, knowing that they have pathogenic E. coli in the herd. If they are considering an autogenous bacterin, or need susceptibility results, we can proceed to further testing of individual isolates.

Image 2– Gallant's Diagnostics: Isolating from the client's samples

Testing Clinical Samples for *E. coli*:

E. coli is a normal inhabitant of the intestinal tract of warm-blooded animals and can be readily isolated. Although usually harmless, some *E. coli* strains carry virulence genes that render them pathogenic and may cause enteric and diarrheal disease, urinary tract infections and septicemia in livestock.

Neonatal diarrhea in pigs and calves is common and the causative agent(s) may include *E. coli*, *Clostridium perfringens*, *Clostridium difficile*, *Salmonella* and/or viruses. So, it is important to determine if the root cause is actually *E. coli*.

E. coli can also be a major cause of postweaning diarrhea (PWD) in pigs and should be suspect. PWD due to *E. coli* is caused primarily by ETEC, a pathotype that is characterized by production of adhesions (F4 or F18) that mediate bacterial adherence to the intestine and enterotoxins (LT, STa or STb) that cause diarrhea (Fairbrother et al., 2005).

In bovine (calves) ETEC and STEC are responsible for diarrhea or hemorrhagic dysentery (Franck et al., 1998). In poultry, ExPEC are associated with colisepticemia or cellulitis (Dho-Moulin & Fairbrother, 1999).

E. coli can also cause disease in many other species including caprines, ovines, cervines and companion animals. Since PCR testing is based on the detection of *E. coli* specific virulence factors, it can also be used to test samples from these species.

Screening for pathogenic *E. coli* and determining the pathotype(s) present is the first step towards resolving *E. coli* mediated disease on a farm. This will enable the attending Veterinarian to make management decisions that could include the use of a commercial or autogenous bacterin product.

Sample Type and Submissions:

Sample types submitted for *E. coli* testing **will not change**. Appropriate samples are intestines, rectal swabs or feces for animals with diarrhea or extraintestinal tissues (heart, liver, lung) from animals with septicemia or localized infections. **A brief history (clinical signs, animal species, age and sample type) is required** on the submission form to guide Lab Technicians in the choice of virulence factors to test and the interpretation of results. **As a reminder, all diagnostic samples are shipped to Units 12 and 13.**

In general, turn-around-time (TAT) for *E. coli* testing at Gallant Labs will shorten with better characterization of *E. coli* isolates. The TAT for primary screening will be 3 - 5 working days. Further 3 - 5 working days may be required if the recovery of pure *E. coli* requires additional isolation steps or if the predominance of a specific pathogenic virotype is low.

Cost of Test:

The cost of the test is affected by the number of samples submitted and the number of virulence factor PCR reactions performed throughout the entire testing procedure. The number of virulence factors included depends on the history of the case (is this a scour problem, a systemic problem, is edema present), the sample type and the set of virulence factors associated with ETEC, EPEC, STEC or ExPEC determinations. Pooling of submitted samples during the initial screening process is one way Gallant endeavours to reduce cost. Therefore, each case is unique, and the cost will depend on the number of individual virulence factor PCR reactions required to complete the case. Please check our website for current pricing.

Please note: (Additional charges may apply for set-up, susceptibility testing, and/or additional screening for other enteric pathogens.)

The benefits of the new PCR test include its ability to quickly screen animal samples for pathogenic *E. coli* and allow for the selection of only those isolates carrying *E. coli* virulence factors for antimicrobial susceptibility and/or inclusion in a herd specific bacterin. This technology enables Gallant to fine-tune strain selection and may even reduce the number of different isolates needed in an autogenous bacterin, which can lower antigen load and costs. For more information regarding Gallant's new PCR testing method please contact Gallant Custom Laboratories at 1-888-838-5223.

References:

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- Franck, Sophia M., Brad T. Bosworth and Harley W. Moon (1998). Multiplex PCR for Enterotoxigenic, Attaching and Effacing, and Shiga Toxin-Producing *Escherichia coli* Strains from Calves. Journal of Clinical Microbiology. 1998 June; 36(6): 1795-1797.
- Dho-Moulin M and Fairbrother JM (1999). Avian pathogenic *E. coli* (APEC). *Veterinary Research* 30: 299-316.
- http://www.ecl-lab.com/en/products/virotyping.asp

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