CONTROL ID: 2021202

TITLE: Application of MIQE Guidelines for Use in a Veterinary Diagnostic Laboratory PCV2 qPCR

ABSTRACT BODY:

Narrative: There is widespread lack of transparency, standardization and assay quality control found in publications that report qPCR assays. The Minimum Information for publication of Quantitative real-time PCR Experiments guidelines (MIQE) provides researchers a set of recommendations for properly reporting qPCR results. The Boehringer Ingelheim Vetmedica, Inc Health Management Center (HMC) recently implemented several MIQE enhancements to the TaqMan real-time PCV2 qPCR to further define limit of quantification (LOQ) and accuracy. Enhancements included linearizing the supercoiled plasmid in the standard curve that can lead to DNA quantity overestimation (1,2) and testing samples in triplicate. The objective of this paper was to evaluate the impact MIQE changes had on a known quantity PCV2 virus. Both the crossing quantity (Cq) and qPCR copy numbers generated for PCV2 controls were compared. All controls were tested with TaqMan real-time PCR reagents using a supercoiled PCV2 10x10 DNA plasmid both provided by Life Technologies Corp., Grand Island, NY. The plasmid was linearized by a Hind III digest kit (Promega corp., Madison, WI) to create qPCR standards. All samples were extracted followed by PCR in triplicate. A LOQ of 3.5 copies was established per published guidelines although they may still be deemed positive. Individual positive extraction control aliquots (n= 162) have been tested since June of 2012. Eighty-sevenwere quantified using the traditional method, prior to implementation of MIQE changes, and 75 following implementation of the changes. The average Cq's and qPCR copies/reaction ± the SE number generated were 24.07±0.80 and 7.58x106 ± 8.08x107, respectively, for the traditional; and 23.84±0.23 and 1.06x104 ± 2.62x103 respectively, for the MIQE implemented method. The actual quantity for the PCV2 virus extraction control was 5.0x103 TCID50 as determined by culture. On average the quantity generated for the PCV2 virus extraction control was 3 logs higher for the traditional method as compared to the improved method. This improved method determined a more accurate estimate of the PCV2 extraction control compared to the traditional method. Practitioners need to be aware of the methods of detection and quantification and limits of detection in their chosen laboratory and take caution when directly comparing qPCR results from various laboratories.

References

1. Hou Y, et al. 2010, Serious overestimation in quantitative PCR by circular (supercoiled) plasmid standard: microalgal pcna as the model gene. Plos One 5(3):e9545.
2. Lin C, et al. 2011, Quantification bias caused by plasmid DNA conformation in quantitative real-time PCR assay. Plos One 6(12):e29101.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021814

TITLE: Initial Evaluation and Validation of High-Throughput (384-well) Real-Time PCR for Testing Bulk Milk Samples

ABSTRACT BODY:
Narrative: Surveillance and preparedness for disease outbreaks is one of the most important missions of veterinary diagnostic laboratories nationally. In particular, PCR-based testing has key advantages for surveillance and outbreak response, including the ability to rapidly scale-up for high-volume, rapid turn-around testing. A California-Wisconsin veterinary diagnostic laboratory partnership was formed to evaluate a 384-sample PCR platform using an endemic virus, *Bovine Viral Diarrhea virus*, and milk as the sample matrix. The approach presents proof-of principle data for "outbreak scalability," with the additional intent to evaluate and validate a test that could be used as a component of routine dairy health management testing. Validation was performed as recommended by the National Animal Health Laboratory Network (NAHLN) Methods Technical Working Group MTWG). Eight BVDV detection methods (four extraction methods and two PCR amplification procedures) were compared. Extraction method evaluations were chosen for equivalency to the method considered by the NAHLN for testing *Foot-and-Mouth Disease virus* in milk, and from approaches used for milk testing by accredited AAVLD laboratories. Analytical sensitivity was quantified using a synthetic plasmid for each combination of extraction, amplification, and detection method. All assays evaluated included an internal control used for detecting PCR inhibition. A panel of BVDV reference strains were used to confirm detection of all BVDV genotypes known to be present in the U.S. Comparison between 96-well and the 384-well platforms was performed for all steps included in the assay validation. An important enhancement was the addition of a visible dye to aid technicians in confirming that assay wells were accurately loaded with both reagent and sample. Milk from a persistently infected (PI) BVDV cow serially-diluted into bulk tank milk showed that detection of a single PI animal in a bulk tank sample to which 1,000 cows contributed. Alternatively, sensitivity was calculated using the number of pounds a PI cow contributed to a bulk milk tank and data suggests that detection for a single animal persistently infected with BVDV is possible up to a 1:10,000 dilution. Individual milk samples and bulk tank milk samples (n=462) of various sizes and origin were used in the field validation testing.
Assay performance was statistically similar between the 96-well and the 384-well platform ($p=0.01$). Overall, the 384-well approach shows promise for not only high-throughput endemic disease testing, but may also provide a feasible candidate for further evaluation by the NAHLN for enhancing national foreign animal disease response testing capacity and capability.

CURRENT CATEGORY/DISCIPLINE: Virology | Epidemiology

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AWARDS:

Trainee Letter:

CONTROL ID: 2016259

TITLE: Rapid Detection of Pathogens from Swine Clinical Samples Using a Broad Spectrum Microbial Detection Array

ABSTRACT BODY:
Narrative: To best safeguard human and animal health requires early detection and characterization of disease events. This must include effective surveillance for emerging infectious diseases. Both deliberate and natural outbreaks have enormous economic and public health impacts, and can present serious threats to national security.
To evaluate the initial utility of a novel and comprehensive microbial detection technology, the Lawrence Livermore Microbial Detection Array (LLMDA) to expedite faster and better detection of emerging and foreign animal disease pathogens, we analyzed a series of swine clinical samples from past disease events. The LLMDA (1) contains probes to detect >8000 species of microbes including 3,856 viral, 3,855 bacterial, 254 archaeal, 100 fungal, and 36 protozoan species that were sequenced through June, 2013. This microarray targets both conserved and unique genomic regions of sequenced microbial strains. The automated data analysis algorithm, Composite Likelihood Maximization, is integrated with a web interface that enables LLMDA data analysis within 30 minutes.
Clinical (serum, oral fluids, tissues and fecal) samples from past disease outbreaks were collected by or submitted to Kansas State University. The samples were shipped to Lawrence Livermore National Laboratory and nucleic acid samples were extracted using Trizol. The samples were amplified using random amplification, fluorescently labeled and hybridized to the LLMDA.
Porcine circovirus 2 (PCV2) and *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV) were the most dominant pathogens detected. We found that oral fluids were a good substitute for serum for pathogen detection. In addition to PCV2 and PRRSV, the LLMDA also detected other previously undetected viral co-infections including *porcine parainfluenza*, *astrovirus*, and *bocavirus* from oral fluid samples. Common bacterial co-infections detected by the LLMDA were *Streptococcus suis*, *Actinobacillus pleuropneumoniae*, *Staphylococcus* sp. and *Enterococcus* sp.
We then compared array results with PCR results in the detection of PCV2 and PRRSV. Initial results showed that the LLMDA detected PCV2 and PRRSV from pig serum samples at Ct of 30 or less. Additional sensitivity testing is under way.
In summary, we have demonstrated that the broad spectrum microbial detection technology, the LLMDA, is sensitive in the detection of known and emerging swine pathogens. It can be used to identify viral and bacterial co-infections, discover unknown pathogen outbreaks, and correlate the effects of microbiome to the health of animals. Its most appropriate, cost-effective application presently is as a secondary diagnostic test to assist further in evaluation of situations where primary syndromic testing does not identify a causative agent.

References
1. Gardner S, Jaing C, McLoughlin K, Slezak T: 2010, A microbial detection array (MDA) for viral and bacterial detection. BMC Genomics 11:668.

CURRENT CATEGORY/DISCIPLINE: Virology | Bacteriology/Mycology

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AWARDS:

Trainee Letter:

CONTROL ID: 2022080

TITLE: Bioinformatics for Improved Pathogen Detection: Maintenance of the Virotype® PRRSV RT-PCR Reagents for Improved Accuracy

ABSTRACT BODY:

Narrative: Accurate pathogen detection is essential in many fields, ranging from infectious diseases diagnostics in humans and animal medicine to pathogen screening for biosecurity. However, the development of tools for specific pathogen detection can be very complex due to the existence of many pathogenic strains, with varying mutations, alongside the ever-present threat of new emerging strains. Typical examples include the *Influenza A virus* and *Porcine Respiratory and Reproductive Syndrome virus* (PRRSV), which can have catastrophic economic consequences for the swine industry. Both *Influenza A virus* and PRRSV have high mutation rates and regional strain variations exist.

Virotype PRRSV NA/EU real-time PCR reagents are designed to detect North American and European PRRSV strains in a multiplex format with an internal positive control. Bioinformatics is used for surveillance of QIAGEN assays. Through the routine use of pathogen genomic characterization, the bioinformatics team can access the success rates of current assay oligonucleotide design, and when necessary identify critical sequences that may require assay is adaptation.

In 2013, diligent bioinformatics alerted critical sequence changes, which might impact the accuracy of the virotype PRRSV NA/EU Reagent. After notification from the bioinformatics team that the reagents were missing strains of the Midwestern region of US (Iowa), in silico PCR was applied to compare virotype PRRSV primer and probe design with PRRSV strains in the database. This analysis allowed for design modification for assay oligonucleotides, which was implemented to maintain accurate detection of the regional PRRSV strains, and maintain detection accuracy of other known strains.

The analytical sensitivity of the modified virotype PRRSV NA/EU oligonucleotides was performed in a translational research lab at Iowa State University (Trujillo). Utilizing purified RNA, from select PRRSV strains, we evaluated several modified oligonucleotides for the virotype PRRSV NA/EU Reagent alongside another commercially available PRRSV detection reagent.

Results show that by utilization of bioinformatics data to aid in assay evaluation and redesign, the modified virotype PRRSV NA/EU Reagent could accurately detect the regional strain and conventional strains. Furthermore, the modified virotype PRRSV NA/EU Reagent demonstrated improved sensitivity of detection as compared to the other commercially available PRRSV detection reagents for the regional isolate (strain Iowa 21).

Academic collaboration and attainment of sequence information for atypical virus isolates coupled with diligent deployment of bioinformatics aided in assessment and successful redesign of oligonucleotides utilized in the QIAGEN virotype PRRSV EU/NA Reagent. Diligent deployment of bioinformatic analysis on a regular basis or in response to a reported outbreak, with new sequences continually being added to the internal database through collaboration will insure assay performance.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2020648

TITLE: An Empirical Approach to Confidence Intervals of Positive and Negative Predictive Values Extrapolated from Case Control Studies

ABSTRACT BODY:
Narrative: The fit of purpose of a diagnostic test can be assessed with a cross-sectional study. Estimates of sensitivity, specificity, prevalence, and positive and negative predictive values (PPV/NPV) can be assessed, as well as the respective 95% confidence intervals (95CI). Unfortunately, cross-sectional study can be difficult to implement and case-control studies are often preferred when assessing the performance of a diagnostic test. Then, the sensitivity and specificity observed are extrapolated to calculate predictive values given different prevalence. This approach provides good point estimates of PPV/NPV but does not allow an easy calculation of the 95CI.
A simulation strategy is proposed to estimate the confidence intervals of PPV/NPV. From the sensitivity and specificity estimates, the bayesian 95CI are calculated. These confidence intervals present the advantage of distributions and therefore can be used to sample from. The sampling outputs are computed with a given prevalence value in order to establish a posterior distribution of the predictive values. The respective 95CI are established. The simulation is repeated for all prevalence values between 0 and 1 with increments of 0.01. All computations are realized in R 3.0.1. The simulation is illustrated using two hypothetical tests (Test 1: Se=99%, Sp=99%; Test 2: Se=95%, Sp=100%) and is evaluated in two populations of different size (Pop. a: 100 infected, 100 healthy; Pop. b: 100 infected, 1000 healthy). Graphical outputs illustrating PPV/NPV and their respective 95CIs in function of the prevalence, sensitivity, and specificity. Test 1 and test 2 are compared and despite the difference between the positive predictive values at low prevalence, large overlapping of their confidence intervals illustrate the importance of considering both the value and the precision of the value before inferring decision upon fit for purpose of the test. Additional comparison illustrates the impact of sample size on the predictive values' confidence intervals.
The empirical method hereby described presents a convenient alternative to obtain 95% confidence intervals of predictive values extrapolated from sensitivity and specificity estimates from case-control studies. This method can be use to compare predictive value before selecting a test over another in a particular population. Alternatively, the simulation model could be reversed and used to infer ideal sample size for obtaining the precision targeted for the PPV/NPV.

CURRENT CATEGORY/DISCIPLINE: Epidemiology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021309

TITLE: Oklahoma Equine Cases Presenting with Clinical Signs of Central Nervous System Disease– 2012-2013

ABSTRACT BODY:
Narrative: The identification of a pathogen underlying equine neurologic symptoms plays an important role in equine and public health. A probable diagnosis can lay the groundwork for risk analysis and suspected diagnosis for equines and other mammals in the geographic vicinity, including humans. This can also facilitate preventive and control measures relevant to the disease. Testing for common agents that underlie equine central nervous system (CNS) disease, however, remained low from 2008-2011 at The Oklahoma Animal Disease Diagnostic Laboratory (OADDL). In an effort to increase testing, the Oklahoma Department of Agriculture, Food and Forestry (ODAFF) subsidized a diagnostic profile for horses

presenting CNS symptoms. This profile included *West Nile Virus* (WNV) and *Eastern Equine Encephalitis Virus* (EEE) by IgM capture ELISA on serum as well as *Equine Herpes Virus 1* (EHV-1) by PCR on whole blood and nasal swab. Supplemental diagnostics for Equine Protozoal Myelitis (EPM), Western Equine Encephalitis (WEE), Venezuelan Equine Encephalitis (VEE), Rabies, or plant toxins was available as fee-for-service to the client. OADDL solicited submissions from Oklahoma veterinarians and attempted to collect demographics, vaccination history, and clinical signs on all cases. A total of 269 Oklahoma-resident CNS cases were submitted from January 2012 through December 2013, with 147 cases meeting the full sample requirements for the subsidized program. The data are presented as a percentage, with positive results over the total number of cases submitted for each test. Twenty-nine percent (69/237) were positive for WNV. All EHV-1 submissions (167 nasal swabs, 190 whole blood samples) were negative and all EEE submissions (213) were negative. Equine Protozoal Myeloencephalitis (EPM) combined IFAT emerged as a common supplemental test request for which 68% (38/56) had a positive titer. Two out of 10 brain specimens tested for rabies virus were positive. An inherent challenge to serological testing of horses with CNS disease is that it only identifies a probable agent, but does not confer a definitive diagnosis. Results may indicate exposure to more than one pathogen. For example, of 5 WNV-positive cases also tested for EPM, 4 were positive for EPM. A low percentage of cases reported current vaccination status at time of submission: 29% WNV, 31% EEE, and 25% EHV-1. This data contributes to surveillance for both common and zoonotic CNS pathogens and promotes awareness among equine veterinarians and owners.

CURRENT CATEGORY/DISCIPLINE: Epidemiology

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AWARDS: AAVLD Laboratory Staff Travel Award

Trainee Letter: AAVLD Staff Significance Role Lenoir.pdf

CONTROL ID: 2022310

TITLE: *Salmonella* spp. Serotypes Isolated from Drag Swabs at a Veterinary Diagnostic Laboratory and Public Health Implications

ABSTRACT BODY:
Narrative: *Salmonella* spp. is an important zoonotic cause of gastroenteritis in humans and animals. Most cases in humans are due to bacterial contamination of food (foodborne illness) and less commonly due to direct contact with affected animals or contaminated water. Many serotypes of *Salmonella* spp. have been documented to cause foodborne disease, with *Salmonella enterica* serotype Enteritidis (SE) from contaminated eggs most commonly reported. Testing of environmental drag swabs from poultry housing facilities is a validated screening tool for SE.
As specified by the FDA under the Egg Safety Rule, the identification of SE from an environmental drag swab results in the holding of eggs until further negative testing is obtained. The identification of other *Salmonella* spp. serotypes may not result in regulatory implication even though other serotypes have been associated with foodborne illness from the consumption of poultry products. The objective of this study was to determine the prevalence of different Salmonella serotypes isolated from drag swabs submitted to the Utah Veterinary Diagnostic Laboratory (UVDL) during a 3-year period.
A total of 868 drag swabs were tested from 2009 through 2011. Of those, *Salmonella* spp. was isolated from 126 swabs (14.5%) using tetrathionate enrichment followed by plating onto Hektoen enteric agar and XLT-4 (Xylose-Lysine-Tergitol 4) agar or MSRV (Modified Semi-Solid Rappaport Vassiliadis) agar. Suspect *Salmonella* spp. colonies were presumptively

identified using an LIA (Lysine Iron Agar) slant and a TSI (Triple Sugar Iron) slant and then were identified to genus using API 20E strips. The *Salmonella* spp. isolates were submitted to the National Veterinary Diagnostic Laboratories (NVSL) for serotyping.
The most common (27/126 = 21%) serotype identified was *Salmonella* Typhimurium. *Salmonella* Kentucky was the second most commonly (23/126 = 18.2%) isolated serotype. *Salmonella* Enteritidis was isolated from 7 (5.6%) drag swabs.
Salmonella Typhimurium is the second most common serotype associated with foodborne illness nationally and was the serotype identified the most commonly at the UVDL from 2009 through 2011. However, its detection in drag swabs does not currently result in federal regulatory action.

CURRENT CATEGORY/DISCIPLINE: Epidemiology | Bacteriology/Mycology

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AWARDS:

Trainee Letter:

CONTROL ID: 2020527

TITLE: Retrospective Testing for the Emergence of *Porcine Deltacoronavirus* in US Swine at Iowa State University Veterinary Diagnostic Laboratory

ABSTRACT BODY:

Narrative: *Porcine deltacoronavirus* (PDCoV) belongs to the order Nidovirales, family *Coronaviridae*, and genus Deltacoronavirus. PDCoV is a single-stranded, positive-sense, enveloped RNA virus containing a genome of approximately 25 kb. PDCoV was first identified in Hong Kong in a surveillance study published in 2012. According to the study, PDCoV HKU 15-44 and HKU 15-155 strains have been present in pigs in Hong Kong since 2009 and 2010 respectively. PDCoV in U.S. swine was identified for the first time in February 2014 and its presence has been reported in several research studies thereafter. Up to May 15, 2014, PDCoV has been detected in 14 U.S. states according to the National Animal Health Laboratory Network (NAHLN) laboratory testing summary from USDA. However, it remains unknown if PDCoV had been introduced to the U.S. earlier than February 2014. The present retrospective study is being conducted to produce evidence of the possible presence of PDCoV in samples submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) between 2012 and 2013. A total of 1,073 samples (fecal samples, environmental samples, and oral fluids) submitted between November 2012 and December 2013 were tested using a PDCoV-specific real-time reverse transcription PCR (rRT-PCR) assay targeting the membrane (M) gene segment. PDCoV M gene was first detected from a fecal sample acquired on 19th August 2013 from a pig in Minnesota with a history of diarrhea but negative for *Porcine Epidemic Diarrhea Virus* (PEDV) by rRT-PCR. Subsequently, PDCoV was detected in samples collected on 29th August 2013 in two additional pigs from Illinois which were also negative by rRT-PCR for PEDV and *Transmissible Gastroenteritis Virus* (TGEV) and PCR for *Lawsonia intracellularis*, and negative by immunohistochemistry for PEDV, group A *rotavirus* and TGEV. Therefore, at this time and with available samples submitted to the ISU-VDL, it can be inferred that PDCoV has been present in US swine at least since August 2013, suggesting it did not emerge in the US at the same time period as PEDV.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021960

TITLE: Molecular Epidemiology of *Porcine Epidemic Diarrhea Virus* in US Swine

ABSTRACT BODY:

Narrative: *Porcine epidemic diarrhea virus* (PEDV) was detected for the first time in US swine in April 2013 and has spread to 30 US states as of May 2014. However, little is known about the molecular epidemiology of PEDVs in US swine temporally and spatially. In this study, we characterized the genetic profile and diversity of PEDVs circulating in US swine since its emergence. Sequencing for PEDV S1 region was chosen, since this region harbors the postulated neutralizing epitopes, and performed on 167 cases among which full-length genomic sequences were determined on 7 cases. Sequences were compared to 11 additional US PEDV strains whose whole genomic sequences were previously reported as well as 216 non-US PEDV strains with sequences available in GenBank. Among the 178 US PEDVs collected from 23 states, S1 sequences of 156 cases from 22 states had 99.0-100% nucleotide (nt) identity to each other, including the PEDVs initially sequenced after the outbreak in April 2013 (hereafter designated as original US strain). In contrast, S1 sequences of the remaining 22 cases from 10 states had only 92.4-93.8% nt identity to the original US strains, while they shared 99.6-100% nt identity to each other (hereafter designated as variant US strain). Sequence alignment showed that all US PEDV variants had the same patterns of nucleotide changes including some deletions and insertions, when compared to original US PEDVs. Whole genome sequencing demonstrated that the variants had 99.6-100% nt identity to each other and 98.8-99.2% nt identity to the original US strains. Phylogenetic analyses using the S1 nucleotide sequences showed that the variant US strains clustered with some PEDV strains reported from China, which were distantly related to the original US strains. When using the whole genomic sequences, the variant strains still formed a cluster distinct from the original US strains, but its relatedness to the cluster of the original strains was not as distant as that observed in S1-gene based dendrograms. We further developed a S1-gene based differential real-time RT-PCR to quickly distinguish the original strain from the variant strain of PEDV. Retrospective testing of over 1,000 archived fecal samples from December 2012 at Iowa State University Veterinary Diagnostic Laboratory revealed that the original US strain was present from April 15, 2013 whereas the variant US strain was present from May 16, 2013. Our study indicates that there are at least two genotypes of PEDV co-circulating in US swine and the two genotypes of PEDV may be derived from different ancestors but would have been introduced into the US concurrently. The data from this study suggest that within each genotype, PEDVs in the US have not undergone significant genetic changes.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS: Graduate Student Oral Presentation

Trainee Letter:

CONTROL ID: 2016977

TITLE: Improved Diagnostic Performance of a Commercial *Anaplasma* Antibody Competitive Enzyme-Linked Immunosorbent Assay Using Recombinant Major Surface Protein 5–Glutathione S-Transferase Fusion Protein as Antigen

ABSTRACT BODY:
Narrative: The current study tested the hypothesis that removal of maltose binding protein (MBP) from recombinant antigen used for plate coating would improve the specificity of a commercial *Anaplasma* antibody competitive enzyme-linked immunosorbent assay (cELISA). The number of 358 sera with significant MBP antibody binding (≥30%I) in *Anaplasma*-negative herds was 139 (38.8%) when tested using the recombinant major surface protein 5 (rMSP5)-MBP cELISA without MBP adsorption. All but 8 of the MBP binders were negative (<30%I) using the commercial rMSP5-MBP cELISA with MBP adsorption, resulting in 97.8% specificity. This specificity was higher than some previous reports, so to improve the specificity of the commercial cELISA, a new recombinant antigen designated rMSP5–glutathione S-transferase (GST) was developed, eliminating MBP from the antigen and obviating the need for MBP adsorption. Using the rMSP5-GST cELISA, only 1 of 358 *Anaplasma*-negative sera, which included the 139 sera with significant (≥30%I) MBP binding in the rMSP5-MBP cELISA without MBP adsorption, was positive. This resulted in an improved diagnostic specificity of 99.7%. The rMSP5-GST cELISA without MBP adsorption had comparable analytical sensitivity to the rMSP5-MBP cELISA with MBP adsorption and had 100% diagnostic sensitivity when tested with 135 positive sera defined by nested polymerase chain reaction. Further, the rMSP5-GST cELISA resolved 103 false-positive reactions from selected sera with possible false-positive reactions obtained using the rMSP5-MBP cELISA with MBP adsorption and improved the resolution of 29 of 31 other sera. In summary, the rMSP5-GST cELISA was a faster and simpler assay with higher specificity, comparable sensitivity, and improved resolution in comparison with the rMSP5-MBP cELISA with MBP adsorption.

CURRENT CATEGORY/DISCIPLINE: Parasitology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021775

TITLE: Possible Direct Transmission of *Histomonas meleagridis* in Peafowl.

ABSTRACT BODY:
Narrative: *Histomonas meleagridis* is a flagellate protozoan organism that causes severe necrotizing typhlitis in chickens and turkeys as part of the condition known as “blackhead disease”. It typically uses the cecal nematode *Heterakis gallinarum* as a vector and reservoir for infection, but direct transmission has been both naturally observed and experimentally induced in turkey poults. While it is commonly recognized that other gallinaceous birds are susceptible to *H. meleagridis* infection, there is only a single report in the veterinary literature describing the disease and its transmission by *H. gallinarum* in peafowl. In that report, transmission of *H. meleagridis* was accomplished by feeding young peafowl embryonated eggs of *H. gallinarum*, and resulted in high morbidity and mortality rates, indicating that peafowl are likely more susceptible to infection than chickens or pheasants and that it poses a significant concern for peafowl producers. A review of the archived cases at the University of Georgia Athens Veterinary Diagnostic Laboratory and the California Animal Health and Food

Safety Laboratory System yielded 4 cases (2 from each institution) of young (1 week old to 19 weeks old) peafowl with gross and histological findings characteristic of *H. meleagridis* infestation, including a bilateral, transmural fibrinonecrotic typhlitis and multifocal necrotizing hepatitis with intralesional trophozoites morphologically consistent with *H. meleagridis*. The 2 cases diagnosed at UGA also had concomitant necrotizing air sacculitis with intralesional trophozoites, and one of these 2 cases had pulmonary granulomas with intralesional *Aspergillus* spp. hyphae. Immunohistochemistry for *Trichomonas* spp., which cross-reacts with *H. meleagridis*, and genotyping for *H. meleagridis* was done to confirm the diagnosis. There was no evidence of *H. gallinarum* infestation in all 4 cases. Infection in these cases may have occurred by 1) ingestion of embryonated *H. gallinarum* eggs; or 2) direct ingestion of *H. meleagridis* from the environment. Direct infection has not been previously documented in peafowl and further tests will be developed in these cases to determine whether lateral infection could have occurred.

CURRENT CATEGORY/DISCIPLINE: Pathology | Parasitology

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AWARDS: Trainee Travel Award|Graduate Student Poster Presentation|Graduate Student Oral Presentation|AAVLD/ACVP Pathology Award

Trainee Letter: AAVLD Trainee Work and Significance Letter.doc

CONTROL ID: 2021265

TITLE: Efficacy of BioMed TF-Transit Tubes in Comparison to Gold Standard BioMed InPouch TF during Transit

ABSTRACT BODY:

Narrative: *Tritrichomonas foetus* (*T. foetus*) is a flagellate protozoan parasite that is the etiologic agent responsible for the severe reproductive disease in cattle known as bovine trichomonosis. New Mexico Department of Agriculture Veterinary Diagnostic Services (NMDA-VDS) has validated a Real-Time PCR assay that was recently discussed in the Journal of Veterinary Diagnostic Investigations by Effinger and Colleagues as the only assay performed by a laboratory (Laboratory F) in their study that had perfect agreement (kappa = 1.0) with the nPCR and subsequent sequencing results they acquired. To maintain this detection level of the *T. foetus* organism for the clients the laboratory serves, NMDA-VDS performed a study to determine the efficacy of the newly offered BioMed TF-Transit tubes in comparison to the gold standard BioMed InPouch transport system while in transport. This study also served to determine the impact on samples of variable transport times with regard to the detection of *T. foetus* by Real-Time PCR.
The transport study was performed by using a pure strain of *T. foetus* (sequenced) to prepare a stock 10-fold serial dilution (neat through 10-6). The BioMed TF-Transit tubes and BioMed InPouch transport system were then inoculated with each dilution series in triplicate with a negative sample included per group (22 samples per collection system per group; 176 total samples). Four groups of side-by-side comparison collection methods were produced including a laboratory control group, a 48 hour transport group, a 72 hour transport group, and a 96 hour transport group. Once inoculated, samples were prepared for transport with logged temperatures throughout transport time. Upon return, each group was processed through specimen receiving for molecular processing following standard diagnostic procedures. The Molecular Biology department performed the NMDA-VDS validated chemical lysis extraction and Real-Time PCR method and added a standard *T. foetus* reference dilution series on each plate for development of a standard curve and limit of detection.

The results of this study provided data toward the acceptance of the BioMed TF-Transit tube as an efficacious transport system and evidence revealed comparable analytical sensitivity when compared to the BioMed InPouch transport system. Additionally, this study yielded valuable information on acceptable transport times from collection date to date receipt in laboratory, with 96 hours being a permissible and evidence-based transport time when utilizing the validated Real-Time PCR method at NMDA-VDS.

CURRENT CATEGORY/DISCIPLINE: Parasitology

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CONTROL ID: 2016563

TITLE: Improvements in *Tritrichomonas foetus* Molecular Diagnostics

ABSTRACT BODY:

Narrative: Bovine trichomoniasis is a venereal disease with worldwide distribution caused by *Tritrichomonas foetus* (*T. foetus*). *T. foetus* is transmitted during coitus and results in infertility, abortion and diminished calf crops. Cows are often able to clear infection but bulls become lifelong carriers. *T. foetus* infection is of significant economic concern to the cattle industry due to economic losses associated with reduced breeding efficiency. There is no effective treatment, and management strategies are currently limited to testing prior to breeding and culling of infected bulls. Two diagnostic testing methods available are microscopic identification in preputial smegma cultures and quantitative polymerase chain reaction (qPCR). Both methods require incubation in InPouch media after collection.
Notable challenges associated with *T. foetus* diagnostic testing include: cost, associated with sample collection in InPouch media, inconsistencies in shipment conditions, incubation time and temperature, incubation time burden, and labor burden of InPouch sample processing. To overcome these challenges, an innovative qPCR that utilizes direct smegma testing was developed, eliminating cost of the InPouch, need for incubation, and decreasing time and labor burden for submitter and laboratory. Smegma is collected into a sample tube and used directly for nucleic acid purification. Thus, without incubation in InPouch media, faster results are enabled, allowing faster movement of animals.
Assay performance was evaluated using 166 bulls, 56 positives and 110 negatives. T.foetus positive or negative status was determined using InPouch culture microscopic identification. Using microscopic identification as the reference test, the TVMDL currently employed qPCR, which utilizes samples cultured in InPouch media, exhibited 95% diagnostic sensitivity and 100% specificity, McNemar's P-value of 0.25. The new, direct qPCR without prior culture exhibited 100% diagnostic sensitivity and 99% specificity, McNemar's P-value of 1.00. No significant difference was observed between each qPCR compared with the microscopic identification. The agreement between the current and new direct qPCR was 98% (kappa=0.95), P-value of 0.125, indicating no significant difference between the two tests. However, the new direct qPCR identified four additional positive animals and Cq values were lower for all positives: 13.6-33.5 for new direct qPCR vs. 18.7-37.4 for current, post-culture qPCR. The new direct qPCR results enabled better data interpretation since all Cq values were outside of the inconclusive and suspect range.
In summary, the new direct qPCR assay offers significant improvements: easier sample collection process, omission of the InPouch cost and incubation time, easier sample processing and nucleic acid purification in the diagnostic lab, faster results and overall lower cost. These

improvements benefit producers, veterinarians, and diagnostic labs in their efforts to control *T. foetus*.

CURRENT CATEGORY/DISCIPLINE: Bacteriology/Mycology

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