TVMDL Syndromic Approach to Bovine Testing

Diagnostic Methodologies

Version 1.0 (2021)

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Analytical Chemistry and Toxicology

Colorimetric Analysis

- **Diagnostic Use:** This is a qualitative, semi-quantitative, and/or quantitative test method used to detect and measure levels of various toxins, such as cyanide, nitrates (e.g. ocular fluid, serum, urine), and chloride (rumen content, feed, water).
- **Testing Procedure:** Colorimetric analysis is based on a chemical reaction when toxins are present in a sample, indicated by a change in color to the test apparatus.
- **Result Reporting:** Results can be qualitative (i.e. presence or absence), semi-quantitative, or quantitative, depending on the target analyte.
- **Special Considerations:** Cyanide in rumen content is very volatile. Rumen content to be tested for cyanide should be frozen immediately upon collection and shipped frozen.

Distillation

- **Diagnostic Use:** Distillation is used to liberate certain compounds [e.g. ammonia (urea or fertilizer poisoning) and petroleum hydrocarbons] from biological samples (e.g. rumen content) to enhance detection and quantitation.
- **Testing Procedure:** Distillation involves boiling samples in water to volatilize potentially toxic substances. The boiling process creates steam, which is then cooled, forming a concentrated distillate. The distillate can be titrated (e.g. ammonia) or extracted and analyzed instrumentally (e.g. petroleum hydrocarbons) for detection and quantitation.
- **Result Reporting:** Results can be semi-quantitative or quantitative. Ammonia results are reported with interpretation. Significance of petroleum results depends on observed clinical signs.
- **Special Considerations:** Ammonia is very volatile and can escape the rumen quickly upon necropsy. Samples should be collected, frozen immediately upon collection, and shipped frozen.

Petroleum Distillation

Petroleum Distillation

Fourier-Transform Infrared Spectroscopy (FTIR)

- **Diagnostic Use:** FTIR is used in the determination of crystalline and molecular composition and the quantification of urolith components, important to establishing the etiology of urolith formation and disease.
- **Testing Procedure:** FTIR uses infrared technology to produce an absorption spectrum across a broad range of infrared wavelengths.
- **Result Reporting:** Results include the type of urolith identified by the absorption spectrum.
- **Special Considerations:** Uroliths should be submitted dry, not in saline or formalin.

Gas Chromatography/Mass Spectrometry (GC/MS)

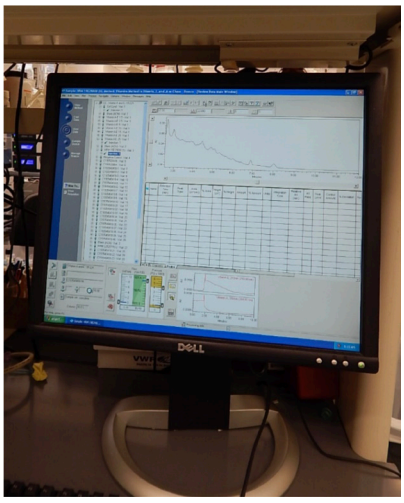
- **Diagnostic Use:** GC/MS is used to identify and quantify a wide range of volatile molecules (e.g. organochlorines, organophosphates, pyrethroids, herbicides, drugs, and hydrocarbons) in biological and non-biological samples. GC/MS is beneficial for screening for a large number of compounds within the same class.
- **Testing Procedure**: Samples are subjected to extraction with organic solvent in a manner that targets the desired class of compounds. The volume of organic solvent is then reduced to concentrate the compounds of interest prior to analysis. Mass spectrometry (MS) is a detection system based on molecular weight of the target analyte (e.g. drug, chemical, mineral, metal), and where applicable, its characteristic fragmentation products. The letters in front of "MS" describe how the sample is introduced into the detector. Gas chromatography (GC) separates molecules using a capillary column and introduces analytes into the mass spectrometer (detector) in a gaseous phase, usually helium. The separated analytes are energized (ionized) for detection, identification and measurement within the MS portion of the instrument. A computerized interface allows a chemist to search the data and evaluate potential molecular matches.
- **Result Reporting:** Results are reported qualitatively (i.e. presence or absence) and quantitatively (e.g. ppb and ppm). Significance is dependent on associable clinical signs.
- **Special Considerations:** None.

Gravimetry

- **Diagnostic Use:** Weight difference before and after sample processing are compared to generate % bone ash as well as tissue dry weight used in vitamin and mineral testing calculations.
- **Testing Procedure:** Tissue as received is accurately weighed to the nearest hundredth of a milligram, then subjected to dehydration (liver, kidney) or ashing (bone) overnight. Once cooled, the remaining material is re-weighed and the difference calculated.
- **Result Reporting:** Bone density (bone ash) results are reported as ash % of bone, representing the mineral content of the bone, with interpretation. Tissue dry matter is reported as dry weight % (minerals) or is used in the calculation of vitamin A & E results.
- **Special Considerations:** None.

High-Performance Liquid Chromatography

- **Diagnostic Use:** HPLC uses ultraviolet and visible spectrophotometry (UV-Vis) to detect and quantify vitamin E and vitamin A in biological samples, and to detect and quantify strychnine in ingesta, bait and urine.
- **Testing Procedure:** HPLC uses liquid chromatography columns to separate components in a mixture. Detection and quantitation is made using UV-Vis detection by comparing the sample data to that of concurrently analyzed reference materials.
- **Result Reporting:** Results are reported quantitatively (e.g. ppm for strychnine, ng/mL, µg/mL or µg/g dry weight for vitamins A&E) with accompanying interpretations.
- **Special Considerations:** Samples for vitamin A or E testing are



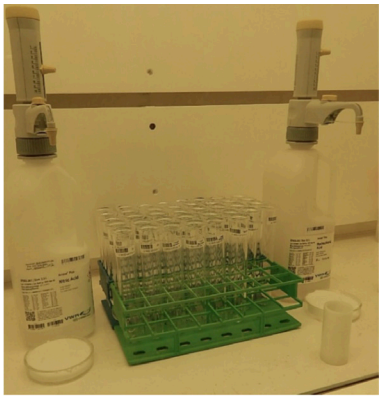
Results screen of vitamin testing via HPLC

sensitive to hemolysis, light and heat. Please pull serum off the clot before shipping and wrap samples in foil or other light-blocking material. Please put liver samples in plastic, then wrap with light-blocking material. Ship all samples for vitamins overnight with cold packs.

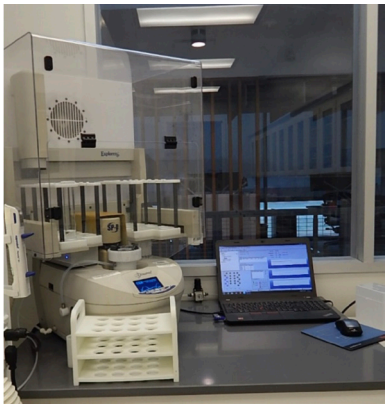
Inductively-Coupled Plasma/Mass Spectrometry (ICP/MS)

- **Diagnostic Use:** ICP/MS is used to detect and quantify various minerals and metals in biological and non-biological samples. ICP/MS is useful for mineral and metal toxicities and for monitoring herd health and individual animal nutrition. At TVMDL, we currently offer testing for each individual metal or mineral as well as for the following panels:
    - Trace Mineral Panel: cobalt, copper, iron, manganese, molybdenum, selenium and zinc
    - Heavy Metal Panel: arsenic, cadmium, lead, thallium and zinc
    - Metal & Mineral Panel: cobalt, copper, iron, manganese, molybdenum, selenium, zinc, arsenic, cadmium, lead and thallium
    - Testing Procedure: Acid-digested, liquified and diluted specimens are introduced into the ICP consisting of an argon plasma that is inductively heated and energized (ionized). This ionized state allows the MS detector to simultaneously measure the ions based on the molecular weights of the specific metals/minerals of interest.
- **Result Reporting:** Results are reported quantitatively (e.g. µg/mL, ng/mL, µg/g dry weight) with accompanying interpretations.
- **Special Considerations:**
    - For accurate serum trace mineral results, please use royal-blue-top (RBT) trace mineral tubes (not powder blue citrate tubes). All other rubber stoppers are processed with zinc stearate and carry some variable zinc contamination. As an alternative, tubes with stoppers made of plastic can be used.
    - For our Trace Mineral Panel or for the Metal & Mineral Panel, please submit either liver (or liver biopsy*) or both whole blood (EDTA) and RBT serum, off the clot and shipped in a second RBT or in a plastic tube.
    - Heavy metal testing on live animals requires whole blood (EDTA preferred). Serum is not a suitable sample for lead, arsenic, cadmium or thallium. Liver is the tissue of choice from a dead animal, although for cattle kidney can be used to confirm lead poisoning.
    - *Liver biopsy suitable for metal & mineral testing consists of at least 50 mg tissue. This is a minimum of 3 tru-cut biopsies, and 4 is better. Send liver tissue in a bag or tube with no added fluid.

Target detection and quantification via ICP/MS



Digestion tubes and acids for sample preparation – metals and minerals via ICP/MS



Microwave Digestion Robot for metals testing via ICP/MS

Kinetic pH/Kinetic Colorimetric Assay

- **Diagnostic Use:** Enzyme kinetics are used to measure acetylcholinesterase activity in whole blood or brain tissue as an indicator of organophosphate or carbamate pesticide poisoning.
- **Testing Procedure:** Samples are homogenized and diluted in a buffer solution that maintains biological activity. Following a baseline measurement, a cholinesterase substrate compound is added and pH or color absorption is measured over a period of time to calculate acetylcholinesterase enzyme activity.
- **Result Reporting:** Results are reported as change in pH per hour or in µmoles/minute/mL with accompanying normal values.
- **Special Considerations:** Samples should be chilled to preserve enzyme activity. Brain tissue for acetylcholinesterase testing is optimally ¼ of a cerebrum. Results for brain tissue are not adversely affected by freezing.

Liquid Chromatography /Mass Spectrometry (LC/MS)

- **Diagnostic Use:** LC/MS is used to identify and quantify a wide range of molecules (e.g. drugs, ionophores, avermectins, anticoagulants, cantharidin, and carbamates) in biological and non-biological samples. LC/MS is beneficial for screening for a large number of compounds, often times at lower concentrations than GC/MS.
- **Testing Procedure:** Samples for LC/MS analyses are initially subjected to extraction procedures to isolate and concentrate compounds in the class of interest. Liquid chromatography (LC) indicates the compounds in the sample are separated over an HPLC column in a liquid phase prior to being energized (ionized) and introduced into the

LC/MS

MS detector. Target analytes are identified based on their molecular weights and the weights of their characteristic fragments. Quantitation is performed by analyzing a series of standard concentrations in similar sample material.

- **Result Reporting:** Results are reported qualitatively (i.e. presence or absence) and quantitatively (e.g. ppb and ppm).
- **Special Considerations:** None.

pH Electrode

- **Diagnostic Use:** Rumen pH is a rough indicator of rumen environment and microbial function.
- **Testing Procedure:** Rumen content is filtered to liberate fluid, which is then subjected to pH measurement using a calibrated meter-probe combination.
- **Result Reporting:** pH value is reported, along with interpretive ranges.
- **Special Considerations:** Rumen pH changes following the animal's death as the microbes continue to process available nutrients. The ranges used for interpretation in the laboratory were developed for shipped-in specimens, and so are somewhat lower than the pH found at necropsy.

Reinsch Test

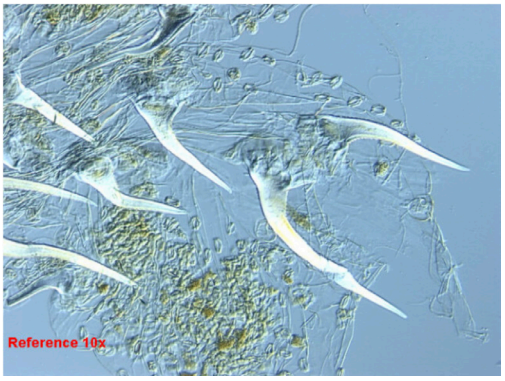
- **Diagnostic Use:** The Reinsch test is used to differentiate whether an arsenic source is an inorganic salt or an organic compound. Typical samples include rumen content and various colored powders.
- **Testing Procedure:** Metallic arsenic is electroplated onto polished copper from an acidified source material.
- **Result Reporting:** Reinsch test results are qualitative – positive or negative.
- **Special Considerations:** The Reinsch test is best used as a follow-up to a positive blood or liver arsenic result.

Toxicology Evaluation (Microscopy)

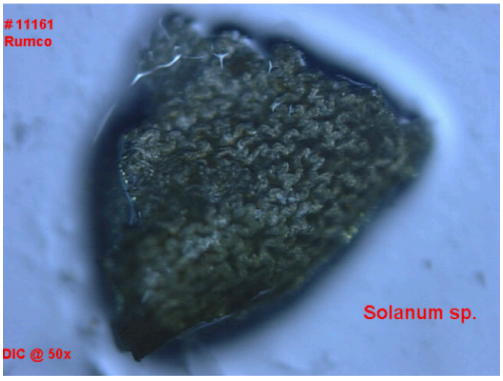
- **Diagnostic Use:** Microscopy is used to identify a wide range of constituents to aid in various diagnoses. Some of these include identification of toxic and non-toxic plant fragments in GI content, identification of cyanobacteria (i.e. blue-green algae) in water, identification of blister beetle parts in stomach content, hay, and alfalfa cubes. Microscopy/visual evaluation is also used to identify submitted toxic plants, bait formulations in GI content/vomitus, and malicious bait samples.
- **Testing Procedure:** Microscopic evaluation is performed at different magnifications and is based on microscopic characteristics and pattern recognition. Identification is made by comparison to voucher and reference source material.
- **Result Reporting:** Results include a description of identifiable components and interpretation to aid in diagnosis when possible.
- **Special Considerations:** Please send up to a quart of rumen content for a more thorough evaluation.

Twin Leaf Senna (*Senna roemeriana*) leaf material from bovine rumen content

Dallisgrass Ergot (*Claviceps paspali*) identified in a hay sample



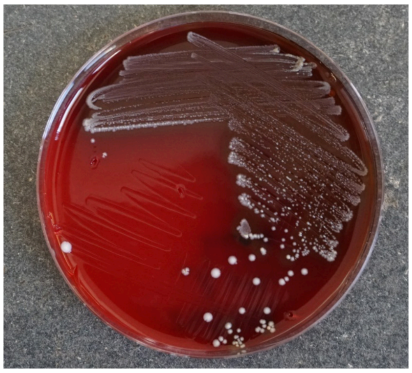
Lantana (*Lantana camara*) leaf material from bovine rumen content



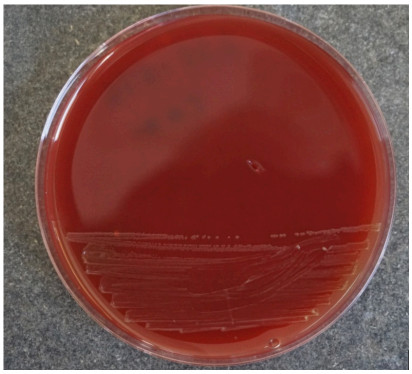
Silverleaf Nightshade (*Solanum elaeagnifolium*) seed fragment from bovine rumen content

Bacteriology and Mycology

Aerobic Culture/ Anaerobic Culture



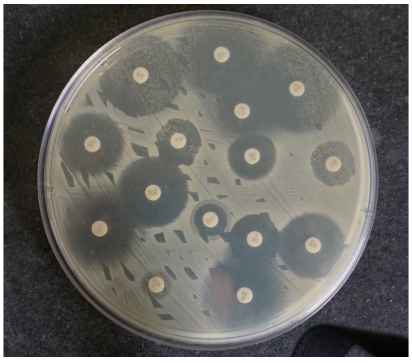
Aerobic Culture



Anaerobic Culture

- **Diagnostic Use:** Bacteria are isolated and identified from wide varieties of clinical specimens (eg. swabs, biopsy sample, milk, etc.). Isolation (successful culture) requires viable bacteria in the sample at the time of attempted culture. Sample quality and handling are important.
- **Testing Procedure**: Samples are streaked onto different agar plates (eg. blood agar, tergitol, XLT4 and PEA) and incubated aerobically and/or anaerobically at 37°C overnight to obtain isolated bacterial colonies. Next, bacteria are identified by different biochemical tests and/or MALDITOF-MS.
- **Result Reporting:** Culture report includes name of isolated bacteria and comments, if applicable
- **Special Considerations:** Specimen requirements vary widely depending on factors such as bacterial pathogens suspected, the species and age of animals, the anatomical site of infections. If case consultation and/or interpretation of culture results are desired, please include adequate signalment, presentation, clinical history, and treatment history with the submission. Sample should be aseptically collected. Do not add formalin, saline, or broth to fresh tissue. Swab samples are encouraged to ship using transport medium.

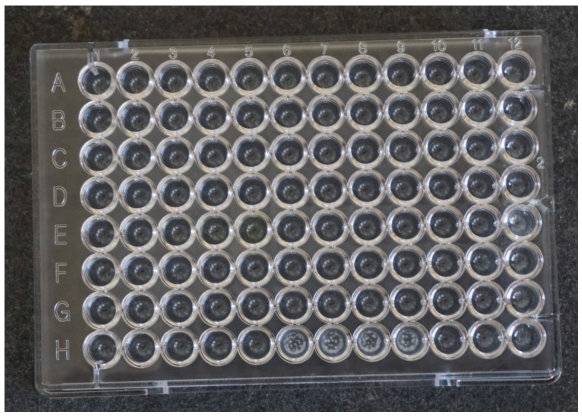
Antimicrobial Susceptibility Testing (AST): Kirby-Bauer (KB)



AST: Kirby-Bauer

- **Diagnostic Use:** Determine the antibiotic susceptibility of bacterial isolates by disk diffusion test
- **Testing Procedure:** Pure bacterial culture is directly mixed into saline or BHI broth and turbidity is adjusted to McFarland 0.5. Using a cotton swab, the bacterial suspension is evenly streaked over Mueller-Hinton/ Mueller-Hinton Chocolate/ Mueller-Hinton with 5% blood agar plate depending on the type of organisms. Once streaking is done, different antibiotic disks are stamped over the agar plate using a stamper. The plate is then incubated in air or CO2 incubator at 37°C for 18-24hours depending on the organism types. The zone diameter of each antibiotic disks are recorded and interpreted according to CLSI guidelines.
- **Result Reporting:** Results are reported as “R”, “I”, “S” and “NI”. R-Resistant, I-Intermediate, S-Susceptible, and NI-No Interpretation.
- **Special Considerations:** Submitted swabs/specimens are cultured to get pure bacterial cultures for antibiotic susceptibility test unless pure culture is provided by the clients.

Antimicrobial Susceptibility Testing (AST): Minimum Inhibitory Concentration (MIC)



AST: Minimum Inhibitory Concentration

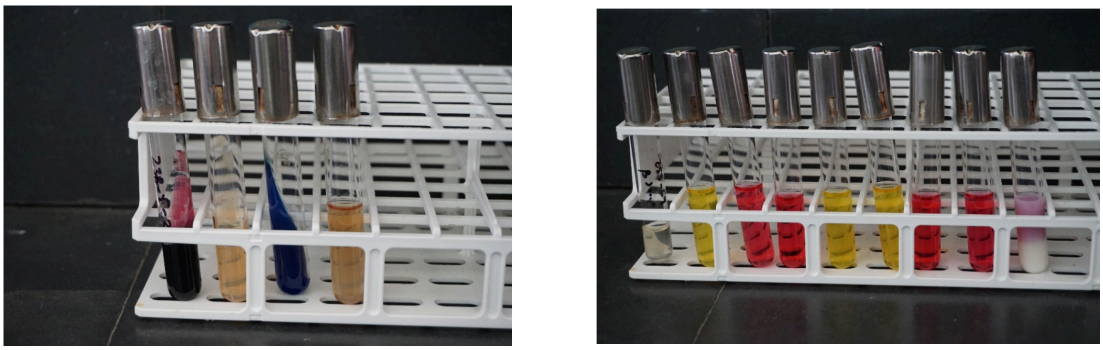
- **Diagnostic Use:** Determine the antibiotic susceptibility of bacterial isolates
- **Testing Procedure:** Pure bacterial culture is directly mixed into saline, and turbidity is adjusted to McFarland 0.5. Depending on the organism types, 1 to 50 µl of the prepared inoculum is transferred into 11ml Mueller-Hinton broth. Finally, using an automatic dispenser 50-100 µl are dispensed into different wells of 96 plate which is coated with different concentrations of different antibiotics. Plate is then sealed using an adhesive tape and incubated in air at 36-38°C for 18-24 hours or up to 5 days depending on the organism types. MIC plate is read by computer software and results are interpreted following CLSI MIC breakpoint guidelines.
- **Result Reporting:** Results are reported as “R”, “I”, “S” and “NI”. R-Resistant, I-Intermediate, S-Susceptible, and NI-No Interpretation.
- **Special Considerations:** Submitted swabs/specimen are cultured to get pure bacterial cultures for antibiotic susceptibility test unless pure culture is provided by the clients.

Bacterial Speciation

- **Diagnostic Use**: Identification of bacterial species isolated from different animal samples.
- **Testing Procedure:** Routine aerobic and anaerobic bacterial cultures are performed on animal specimens to isolate bacteria. If pure bacterial cultures are submitted no further isolation is required. The pure bacterial cultures are then tested using different biochemical tests and/ MALDI-TOF mass spectrometry depending on the organisms types to know the bacterial species. If speciation by these two methods fail, DNA is extracted for 16sRNA PCR and DNA sequencing. The DNA sequences are then analyzed by bioinformatics software to identify the species.
- **Result Reporting**: Name of the bacterial organisms
- **Special Considerations**: Submitting samples must be shipped to TVMDL following proper sample submission guidelines (eg. Sample should be aseptically collected. Do not add formalin, saline, or broth to fresh tissue.)


Bacterial Identification

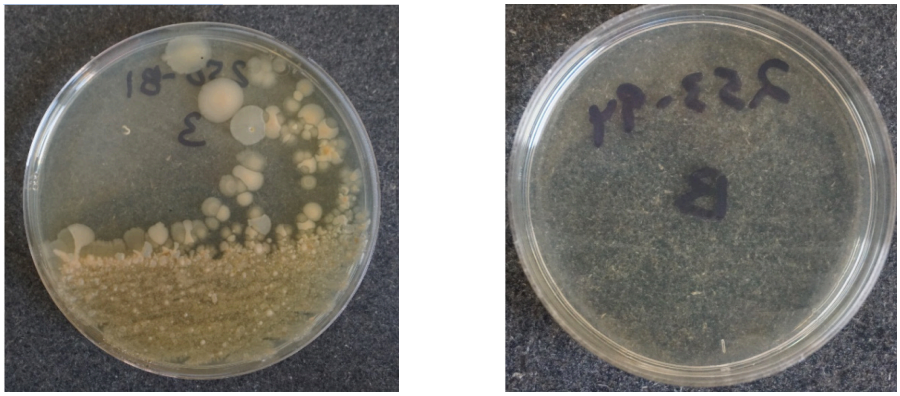

Bacterial Identification



Bacterial Identification

Brucella Culture

- **Diagnostic Use:** Detection of *Brucella spp*. by culture.
- **Testing Procedure:** Samples are streaked onto different agar plates (eg. blood agar, tergitol, XLT4 and PEA, Brucella inhibitory and Brucella plates) and incubated in CO2 incubator at 37°C for 7 days to obtain isolated bacterial colonies. Next, bacteria are identified by different biochemical tests and/or MALDI-TOF.
- **Result Reporting**: Name of the *Brucella* organism/Negative *Brucella* culture
- **Special Considerations:** Ship samples using cold packs.



Brucella + Campylobacter Culture

Campylobacter Culture

Campylobacter Fetus Culture

- **Diagnostic Use:** Detection of *Campylobacter fetus* by culture.
- **Testing Procedure**: For isolation of *Campylobacter fetus* from reproductive samples, clinical specimens (eg. Preputial washings, semen, fetal stomach content and vaginal mucus) are streaked onto Campylobacter medium. Plates are then incubated in a jar containing a microaerophilic gas pack for 5 days at 37° C. Suspected colonies of *Campylobacter fetus* are screened by different biochemicals, motility test and no growth in air. Isolates are confirmed as *Campylobacter fetus* using specific PCR.
- **Result Reporting**: *Campylobacter fetus* or negative culture of *Campylobacter fetus*
- **Special Considerations:** Sample requirements- Fetal stomach content, fetal lung, preputial

washing and cervical mucus are the preferred specimens for Campylobacter fetus. Preputial washings and cervical mucus should be submitted in Campylobacter transport medium (e.g. Amies with charcoal)

Campylobacter Jejuni Culture

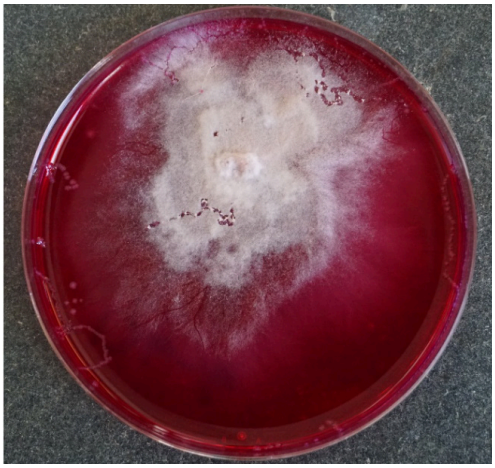
- **Diagnostic Use:** Detection of *Campylobacter jejuni* by culture
- **Testing Procedure:** For isolation of *Campylobacter jejuni*, fecal and milk samples are streaked onto Campylobacter medium. Plates are then incubated in a jar containing a microaerophilic gas pack for 3 days at 37° C. Suspected colonies of *Campylobacter jejuni* are screened by different biochemicals, motility test and no growth in air. *Campylobacter jejuni* is confirmed either by Hippurate test, MALDI-TOF, susceptibility to nalidaxic acid and cephalothin, and PCR.
- **Result Reporting:** *Campylobacter jejuni* or negative culture of *Campylobacter jejuni*
- **Special Considerations:** Sample requirements- intestine, rectal swab, feces, fetal stomach contents, fetal lung, fetal liver, placenta, vaginal discharge and milk

Direct Smear Exam

- **Diagnostic Use:** Identification of *Dermatophilus sp*. and *Campylobacter sp*.
- **Testing Procedure:** Thin smears of scabs/exudates are made on glass slide. Methanol is then used to fix. Next, Giemsa strain is poured on the slides and let it stay for 30-60 minutes. The slides are then examined under the microscope to see the unique appearance of *Dermatophilus*: thickened, branching filamentous rods/cocci looks like railroad tracks. *Campylobacter* can be demonstrated in direct wet mount of stomach content using phase contrast microscopy. This is routinely done for caprine/ovine fetal stomach content.
- **Result Reporting:** *Dermatophilus sp/ Dermatophilus sp* culture negative, *Camphlobacter sp/ Camphlobacter sp* culture negative
- **Special Considerations:**
  - Sample requirements for *Dermatophilus sp* - Scabs and exudates from suspect lesions are the preferred samples
  - Sample requirements for *Campylobacter sp*- fetal stomach content

Direct Smear Exam (Stain)

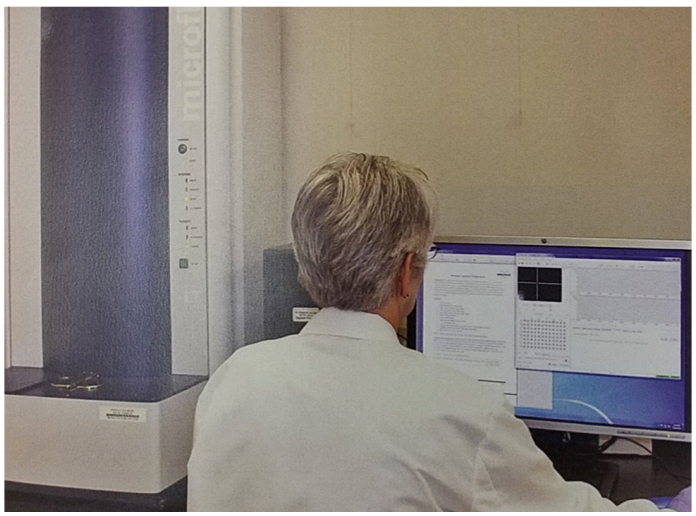
Fungal culture


Fungal Culture

- **Diagnostic Use:** Isolation of fungal organisms of veterinary significant
- **Testing Procedure**: Samples are streaked onto Mycobiotic, Sabouraud, Potato Dextrose agar and Blood agar plates depending on the type of fungal organisms to be identified. Plates are then incubated at room temperature for at least 2 to 3 weeks. A total 6 weeks incubation is required for some systemic fungus like, *Coccidiodes, Histoplasma, Blastomyces, Sporothrix, Phythium and Cryptococcus*. Lectophenol cotton blue staining method is used to examine the fungal culture under the microscope. Based on the morphology of the fungal colonies, organisms are identified and reported in the result section.
- **Result Reporting:** Name of the fungal organisms.
- **Special Considerations:** Whenever possible, submit the actual clinical specimen, as DTM plates, jars, or slants, are often non-diagnostic

Matrix Assisted Laser Desorption/Ionization – Time of Flight (MALDI-TOF)

- **Diagnostic Use:** Identification of bacterial and/or fungal isolates grown in the laboratory
- **Testing Procedure:** Pure bacterial culture is smeared onto a target plate. One microliter of matrix consisting of α-Cyano-4-hydroxycinnamic acid (CHCA) is added onto the target. Next, the target plate is inserted into the plate chamber to perform the analysis. Once analysis is done, the program provides the name of the organism with a confidence score.
- **Result Reporting:** Name of the organism
- **Special Considerations:** Identification of new isolates is possible only if the spectral database contains peptide mass fingerprints of the type strains of specific genera/species/subspecies/strains.

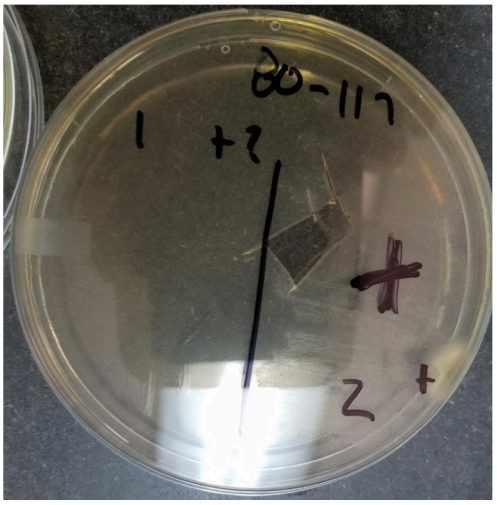

MALDI-TOF

Milk Culture

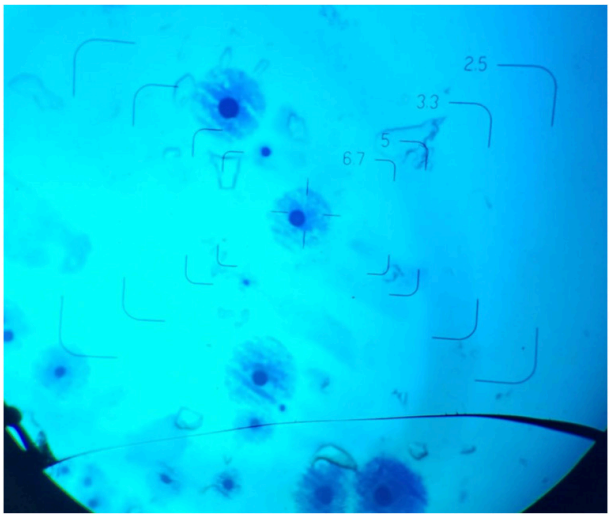
- **Diagnostic Use:** Isolate bacteria from milk samples from patients with suspected, subclinical, or clinical mastitis or bacterial contamination
- **Testing Procedure:** Sterile swab is used to soak milk samples. Samples are then streaked onto different agar medium (eg. blood agar, tergitol, XLT4 and PEA) and incubated aerobically and/or anaerobically at 37°C for overnight to get isolated bacterial colonies. Next, bacteria are identified by different biochemical tests and/or MALDI-TOF.
- **Result Reporting**: Name of the organisms
- **Special Considerations:** Instead of using whirlpaks or zip top bags, send milk samples in sterile tubes with cold packs. TVMDL does not test specimens for food safety or potability. This culture is performed for the purpose of detecting mastitis pathogens only. Raw milk can contain a wide variety of human pathogens that may not be detected by this testing.

Mycoplasma Culture

- **Diagnostic Use:** Isolation and identification of *Mycoplasma* species
- **Testing Procedure:** Using a sterile swab, samples are inoculated directly onto mycoplasma agar and into mycoplasma broth. Next, plate and broth are incubated at 37°C in 5-10% CO2 for a week. Plates are examined daily under dissecting microscope for typical mycoplasma colonies (fried egg). If no colonies are observed after 7 days, the broth is subcultured onto mycoplasma agar, and then incubated for an additional week before being declared as “negative”. Confirmation of *Mycoplasma* is done by PCR when deemed necessary. *Mycoplasma bovis* can be confirmed using Real-Time PCR at the TVMDL
- **Result Reporting:** *Mycoplasma sp/* negative culture of *Mycoplasma sp*
- **Special Considerations:** None

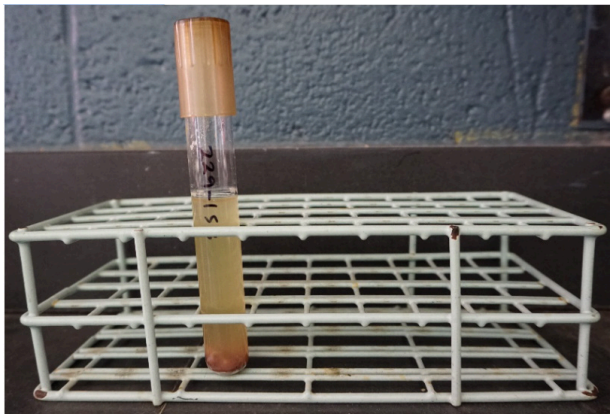


Positive Mycoplasma spp culture of bovine joint sample



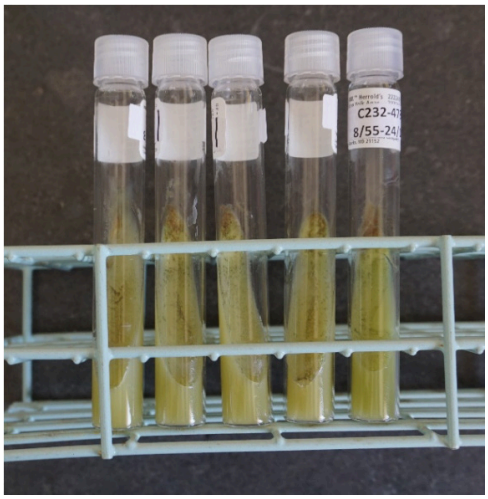
Isolates from the same culture stained and examined via light microscopy

Salmonella Culture


Salmonella Culture

- **Diagnostic Use:** Isolation of *Salmonella* from animals, feed/environmental and water samples
- **Testing Procedure:** Depending on sample types, different steps are followed to isolate *Salmonella*. In general, samples are streaked onto tergitol and XLT4 agar plates and also inoculated into two selective enrichment broths: tetrathionate broth containing iodine and R-10 broth. Plates and broth are incubated in air at 37°C for 1 to 3 days. *Salmonella* produces purple, black colonies on XLT4 agar, and purple, red or clear colonies on tergitor agar. Suspected *Salmonella* colonies are confirmed by different biochemical tests and MALDI-TOF.
- **Result Reporting:** *Salmonella*/Culture Negative of *Salmonella*
- **Special Considerations:** *Salmonella* isolates are forwarded to National Veterinary Service Laboratories, Ames, Iowa for serotyping as needed.

Solid Culture


Solid culture for Mycobacterium

- **Diagnostic Use:** Identification of *Mycobacterium avium* subspecies *paratuberculosis*
- **Testing Procedure:** Two grams of feces is added to 35 ml of sterile water followed by centrifugation. Five ml of supernatant is then transferred into HPC BHI broth. Incubate the broth at 37°C for 18-24 hours. Pellet from the broth is resuspended into 1 ml of Jhone's antibiotic mixture. A 25 µl of the resuspended mixture is inoculated into Harold's egg yolk agar slant containing mycobiocin J followed by incubation at 37°C up to 16 weeks. The slant is observed every 2 weeks for 16 weeks to see the growth of any suspected *Mycobacterium avium* subspecies *paratuberculosis*. Acid-fast staining and PCR are performed to confirm the result.
- **Result Reporting:** *Mycobacterium avium* subspecies *paratuberculosis* / Culture negative *Mycobacterium avium* subspecies *paratuberculosis*
- **Special Considerations:** Testing requires a refrigerated or frozen fecal sample (at least 2 gram)

Special Staining (Acid Fast, Giemsa, Gram)

Acid Fast Staining

- **Diagnostic Use:** Acid-fast staining is used to demonstrate acid-fast organisms such as *Mycobacteria* and *Nocardia* from cultures or clinical specimens such as bacterial colonies or feces
- **Testing Procedure:** Bacterial smear into a slide is prepared by suspending a small amount of bacterial colony in a drop of sterile saline. In case of clinical specimen, such as feces, a

thin smear is prepared using slide-on-slide technique. The smear slide is then stained with TB Kinyoun Carbolfuchsin, 1% sulfuric acid and Methylene Blue following standard laboratory protocol. Acid-fast organisms are identified by observing red or pink stain under microscope.

- **Result Reporting:** Acid-Fast Stain positive / negative
- **Special Considerations:** The preferred specimen is a pure bacterial culture. Clinical specimens, such as feces, may also be appropriate for direct examination. Tissue samples with a request for acid-fast staining must be processed and stained by the Histopathology section.

Giemsa Staining

- **Diagnostic Use:** Identification of *Dermatophilus* sp.
- **Testing Procedure:** Thin smears of scabs/exudates are made on glass slide. Methanol is then used to fix the smear followed by air drying. Next, Giemsa strain is poured into the slides and let it stay for 30-60 minutes. The slides are then examined under the microscope to see the unique appearance of *Dermatophilus*: thickend, branching filamentous rods/cocci looks like railroad tracks.
- **Result Reporting:** *Dermatophilus sp*/ *Dermatophilus sp* culture negative
- **Special Considerations:** Sample requirements for *Dermatophilus sp* - Scabs and exudates from suspect lesions are the preferred samples

Clinical Pathology and Endocrinology

Blood Smear

- **Diagnostic Use:** Evaluation of freshly prepared blood smears to determine WBC differential percentages, review RBC and WBC morphology, providing platelet estimate, and scan for hemoparasites (i.e. *Anaplasma* or *Babesia* spp.).
- **Testing Procedure:** A drop of blood is placed on a glass slide and a thin blood smear is prepared. After the slide has dried completely, it is stained with a Wright-Giemsa stain. The slide is then microscopically reviewed by a highly trained clinical pathology technician. Clinical pathologists may review slides at the request of the submitter or the technician.
- **Result Reporting:** Qualitative reporting of RBC, WBC, and platelet counts and morphology, and presence or absence of hemoparasites (see Hemoparasite exam below).
- **Special Considerations:** Submission of freshly prepared blood smears improves the results. This test is more expensive than a CBC (see above) and should only be used when EDTA whole blood is unavailable (clotted, small animals, etc.).

Blood smears for microscopic evaluation

Chemiluminescence

- **Diagnostic Use:** Measurement of various molecules in the blood (serum): mostly hormones (thyroid, adrenal and sex steroids etc.), but not only (B12 and folates, TLI, etc.).
- **Testing Procedure:** Serum is analyzed in an Immulite 2000 or 2000Xpi by an immunoassay. The molecule of interest (analyte) is recognized by specific antibodies (part of the reagent), and this analyte is then quantified by a chemiluminescent tracer (part of the reagent as well).
- **Result Reporting:** Results are quantitative and provided as a concentration measurement of the analyte. If available for this analyte in the species of interest, reference intervals or interpretation thresholds are provided.
- **Special Considerations:** These assays should be performed on serum (do not send serum on red blood cells/clot, as hemolysis may interfere), typically sent overnight with a cold packs. Some molecules (steroids) are less fragile than others (proteic hormones), which conditions the tolerance in shipping delays.

Chemistry Profile

- **Diagnostic Use:** To provide the results of numerous blood tests which will aid the clinician in determining the source(s) of the illness. It is also used for general health screenings during regular check-ups. Tests include organ function and injury analytes, electrolytes, proteins, etc. Full test lists are available on our website.
- **Testing Procedure:** Testing is performed on an automated analyzer, which can accept various sample types such as serum, plasma, and urine, depending on the test. Spectrophotometry

is the main methodology used for quantifying target molecules.

- **Result Reporting:** Results are quantitative and provided as a concentration measurement of the analyte. Results determined by the analyzer are automatically transmitted to our reporting software, which are then reviewed and distributed by trained staff. Reference intervals and helpful comments are provided depending on species.
- **Special Considerations:** Please visit our website to find recommended sample type and handling requirements for the various tests.

Chemistry Profile - Loading samples into the analyzer

Chemistry Herd Metabolic Profiling

- **Diagnostic Use:** Metabolic profiling, when paired with proper animal selection and correct sample handling, is used to evaluate population level metabolic health and disease risk. Is can also be utilized to provide information on herd nutrition status.
- **Testing Procedure:** TVMDL herd metabolic profiling is performed as a series of specific analytical tests run on a population of at least 8 apparently healthy animals. It is used as a herd-level, rather than individual animal-level, diagnostic evaluation. Blood is taken from each animal and allowed to sit at room temperature for approximately 30 minutes to one hour. Samples are separated into representative population groups based on parity, maturity, lactation stage, or pen. Centrifugation is used to separate the serum from the blood cells and clot and the serum is removed for testing on a diagnostic chemistry analyzer. Each individual animal's results are reviewed for abnormalities and may be retested to verify results. Testing results are then entered into a data spreadsheet by population/group and a group average is calculated for each analyte.
- **Result Reporting:** Results are quantitative and provided as a concentration measurement of the analyte. Results determined by the analyzer are automatically transmitted to our reporting software, which are then reviewed and distributed by trained staff. The spreadsheet for group and lactation stage mean results is created and distributed separately.
- **Special Considerations:** Clinically abnormal animals should not be used for herd metabolic profiling. A minimum of 8 animals is required to qualify for profiling. A minimum of 10 animals is required for the generation of a group mean spreadsheet. Allowing serum to sit on the clot for a prolonged amount of time can result in low glucose and calcium values and an increase in phosphorous. Hemolysis can interfere with some analytes and should be avoided.

Herd Metabolic Profiling Submission – Sample preparation

Transitional

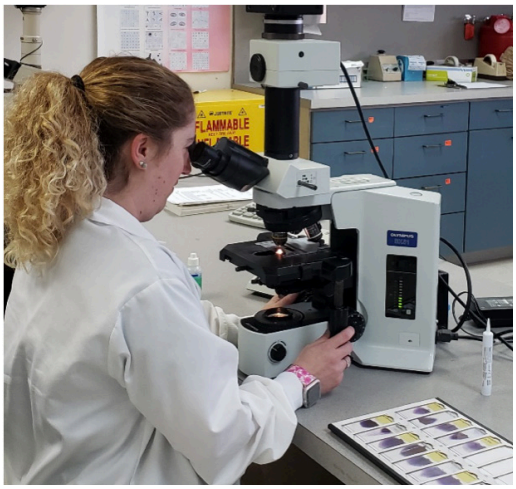
Accession: | Vet: | Sent: 8/30/2019

COW ID	CA mg/dL	PHOS mg/dL	MG mEq/L	ALB mg/dL	UREA mg/dL	GLU mg/dL	CHOL mg/dL	NA mEq/L	K mEq/L	CL mEq/L	NEFA mEq/L	HBA mmol/L	HEMOLYSIS
1148	8.17	5.8	1.8	3.1	16.0	59	68	143	4.2	101	0.31	0.99	N
1159	8.50	7.2	2.0	3.4	17.5	59	71	140	4.4	100	0.36	0.78	N
1190	7.23	6.4	1.6	3.2	15.4	51	72	142	4.0	103	0.53	1.79	N
2386	8.19	5.6	1.8	3.2	14.8	49	98	139	4.1	101	1.31	1.49	N
2775	9.01	7.1	2.0	3.3	13.2	63	91	142	4.4	101	0.13	1.02	N
1068	8.88	7.5	2.1	3.8	17.7	55	70	144	5.1	106	0.29	0.69	N
5079	7.58	6.2	2.2	3.2	13.0	67	68	141	4.0	103	0.30	0.54	N
5088	7.48	6.5	2.2	3.1	16.4	59	70	140	4.3	103	0.62	1.41	1+
5136	8.87	6.9	2.0	3.2	11.6	63	81	140	4.2	102	0.25	0.78	1+
6774	6.18	6.6	2.6	3.4	19.9	62	54	140	4.0	107	0.54	0.60	1+
9019	13.02	7.1	3.0	3.5	16.7	75	67	143	3.9	108	0.16	0.37	1+
9967	8.28	8.0	2.5	3.6	14.4	51	75	143	4.5	105	0.33	1.13	1+
MEAN	8.45	6.7	2.2	3.3	15.6	59	74	141	4.3	103	0.43	0.96	
REF MEAN	9.00	6.0	1.8	3.3	13.0	58	81	142	4.5	101	<0.6	NA	TECH INITIAL:
SD	1.00	1.1	0.3	0.3	3.6	7	42	4	0.5	4			rs

Herd Metabolic Profiling Spreadsheet

Complete Blood Count

- **Diagnostic Use:** Evaluation of blood with both automatic and microscopic methods to evaluate red blood cells, white blood cells, platelets, fibrinogen, plasma protein, etc.
- **Testing Procedure:** EDTA whole blood is analyzed in an instrument designed for this purpose to obtain red blood cell counts, white blood cell counts, platelet counts, and red blood cell indices (see results reporting). A fibrinogen concentration is measured via heat precipitation. Lastly, Blood smear and Hemoparasite exams (details below) are included with every CBC.
- **Result Reporting:** Numerical reporting of: WBC, WBC differential, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, and fibrinogen. Qualitative reporting of RBC, WBC, and platelet morphology, and presence or absence of hemoparasites (see Hemoparasite exam below).
- **Special Considerations:** Submission of freshly prepared blood smears improves the results of the CBC. Keep whole blood samples cold, especially in summer months.



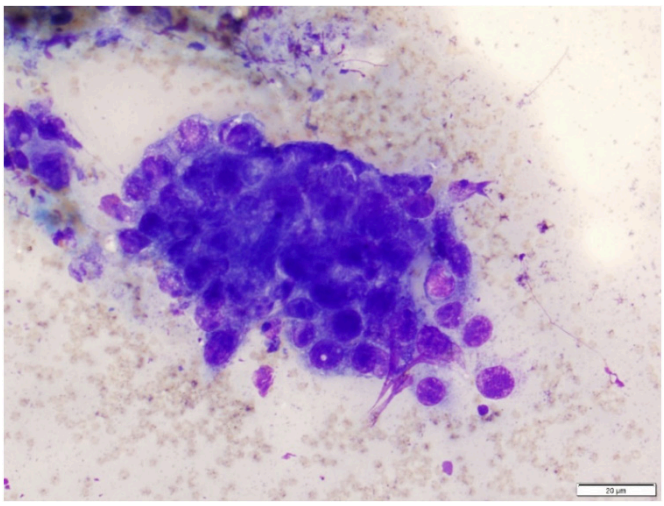
Complete Blood Count

Cytology

- **Diagnostic Use:** Evaluation of body fluids, impression smears, or fine needle aspirates to characterize underlying pathology.
- **Testing Procedure:** If slides are submitted (i.e. fine needle aspirates or impression smears), they are stained with Wright-Giemsa and examined microscopically. Body fluid cytologic examination (i.e joint fluid, ascites fluid, uterine flush) includes gross examination, protein concentration, cell counts (when possible), and microscopic evaluation of direct, sedimented, and cytocentrifuge

preparations. Microscopic evaluations are performed by clinical pathologists.

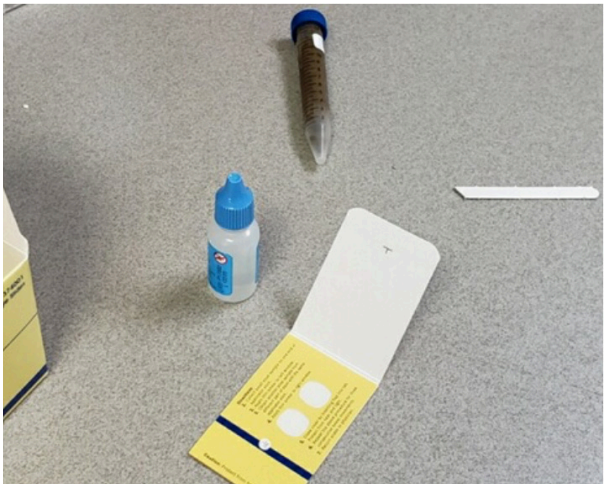
- **Result Reporting:** Cytology reports include full microscopic description, cytologic interpretation, and clinical comments.
- **Special Considerations:** Providing pertinent clinical history and lesion descriptions on the submission form greatly improve the results provided by the clinical pathologist. Slides should be kept out of the refrigerator and protected from formalin, formalin fumes, cold packs, and extreme heat.



Fine needle aspirate of a bovine mandibular lymph node with a metastatic squamous cell carcinoma.

Guiac Fecal Test

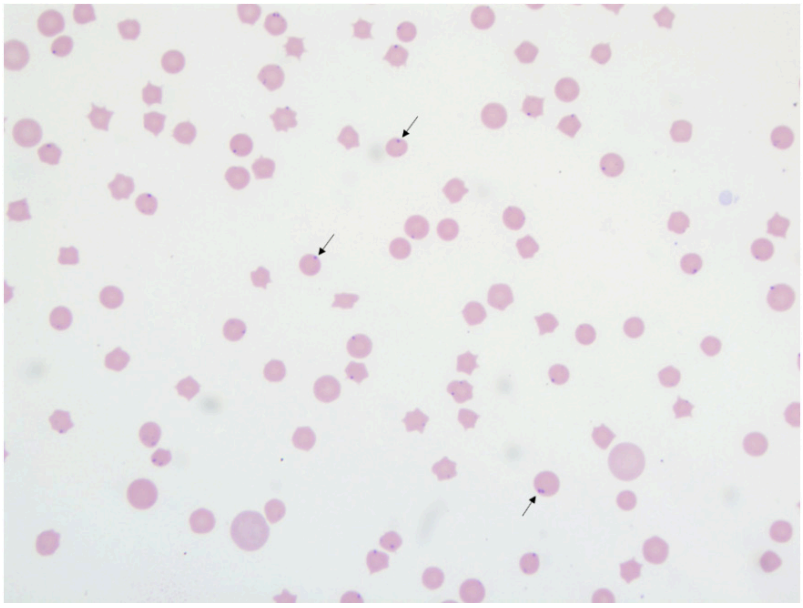
- **Diagnostic Use:** This test detects fecal occult blood which can indicate gastrointestinal bleeding.
- **Testing Procedure:** A small amount of fecal material sampled from two different locations of fecal ball is applied to 2 paper windows on a card. The paper contains guaiac which when hydrogen peroxide is applied, will turn a blue color if blood is present.
- **Result Reporting**: Results are reported as positive (blood present) or negative (no blood present in tested sample).
- **Special Considerations:** A negative result does not rule out a GI bleed. Anti-inflammatory drugs may cause false positives.



Fecal Occult Blood Testing – Guiac Fecal Test

Hemoparasite Exam

- **Diagnostic Use:** Evaluation of freshly prepared blood smears for hemoparasites (i.e. *Anaplasma* or *Babesia* spp.).
- **Testing Procedure:** A drop of blood is placed on a glass slide and a thin blood smear is prepared. After the slide has dried completely, it is stained with a Wright-Giemsa stain. The slide is examined at varying magnifications for parasites by a highly trained clinical pathology technician.
- **Result Reporting:** Results are reported as "No hemoparasites seen" or by the name of parasite seen, i.e. "*Anaplasma marginale*" or "*Babesia sp*. seen". Export reports contain a comment regarding the specific hemoparasite species required on export test forms.
- **Special Considerations:** A negative hemoparasite exam does not rule out hemoparasites and PCR or serological assays may be warranted in some cases.

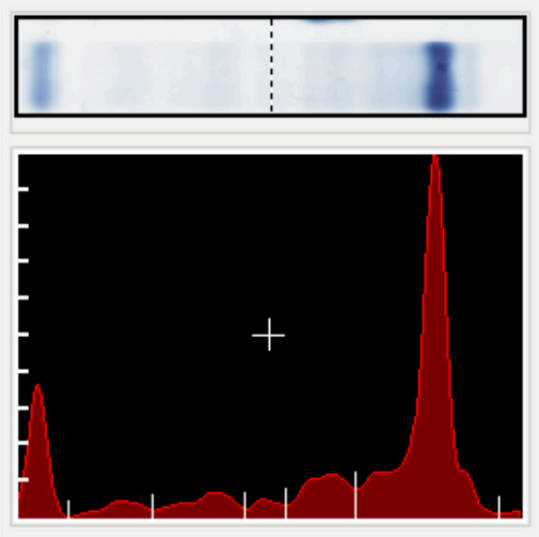


Bovine blood smear. Marked anemia with many Anaplasma marginale organisms (arrow).

Pregnancy Specific Binding Protein (PSBP) ELISA

- **Diagnostic Use:** To provide a "yes" or "no" answer to the pregnancy status in the bovine (cow), ovine (sheep), or caprine (goat) species.
- **Testing Procedure:** The methodology for this test is ELISA (Enzyme-Linked Immunosorbent Assay) which excels in rapid, repeatable results and is especially useful for larger batches of samples (i.e. herd testing).
- **Result Reporting:** Most results provided will be "Open" or "Pregnant" with a rare "Recheck" indicating levels are not elevated enough to be classified as pregnant, but evidence of pregnancy is present and to re-check levels at a later date.
- **Special Considerations:** Bovines can be tested starting at greater than 28 days bred while ovine and caprine should be greater than 30 days bred.

Serum Protein Electrophoresis



Serum Protein Electrophoresis (SPE) Tracing

- **Diagnostic Use:** Various disease states or conditions alter the pattern of migration of proteins in a gel electrophoresis. Hyperglobulinemia can occur with acute and chronic inflammation or some neoplasms. Protein electrophoresis separates the albumin and various globulin fractions and provides useful diagnostic information as to a possible cause for the hyperglobulinemia.
- **Testing Procedure:** Serum is placed on a gel medium and an electrical current is passed through the gel. The various proteins migrate according to their electrical charges and size and a tracing is produced that gives a visual pattern of each protein fraction.
- **Result Reporting:** Results are reported quantitatively as albumin and individual globulin fractions (alpha 1, alpha 2, beta 1, beta 2, and gamma) in mg/dl. A tracing and clinical pathologist's interpretation is included with each report.
- **Special Considerations:** Avoid hemolysis as an artifactual elevation can occur in the alpha and beta regions. Lithium heparin plasma can be used but this sample type results in an artifactual increase in the beta 2 region due to the presence of fibrinogen.

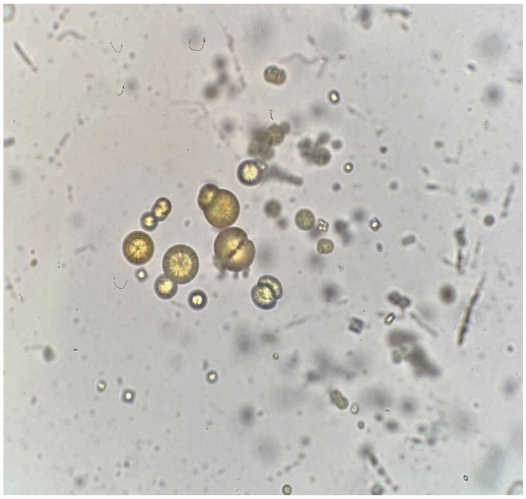
Urinalysis

Urinalysis

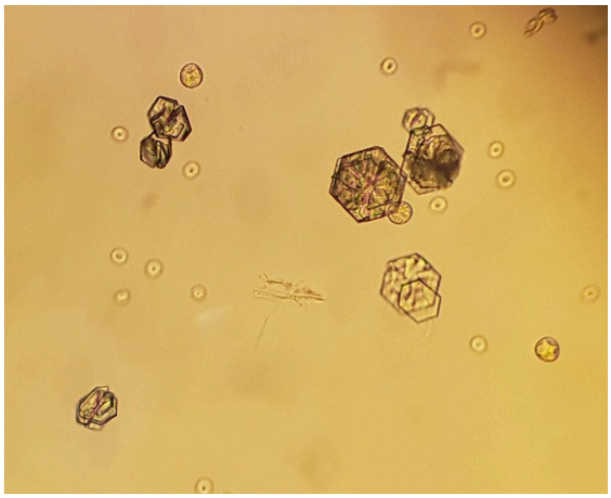
- **Diagnostic Use:** A urinalysis is used to detect and manage a wide range of disorders, such as urinary tract infections, kidney disease and diabetes. This test involves checking the appearance, concentration and content of urine by chemical dipstick methods and microscopic examination on the sediment.
- **Testing Procedure:** This test involves checking the appearance, concentration and content of urine by chemical dipstick method and microscopic examination.
- **Result Reporting:** Reported results are color, character, specific gravity, glucose, ketones, bilirubin, and protein from the chemical strip as well as microscopic findings - quantitative WBC, RBC, epithelial cells (differentiated to squamous and urothelial (transitional)), casts, crystals, and bacteria on the sediment.
- **Special Considerations:** Urine pH can change with sample age and chemical and microscopic results can be adversely affected. Bacterial overgrowth commonly results increased pH which can cause crystal formation and crystal degradation in vitro. Urinalysis is based on a standard sample size of 3 mL. Samples should be collected aseptically and placed in a sterile container then shipped overnight on cold-packs for best results.

Urinary Crystal Identification

- **Diagnostic Use:** Crystalluria can be present with uroliths and can provide information regarding the type of urolith present. Reports include recommendations regarding possible dietary restrictions and medications.
- **Testing Procedure:** The urine sediment is examined for the presence of urinary crystals such as struvites, calcium oxalates, calcium carbonate, etc. Crystals that can be seen in pathological conditions such as ammonium urates and calcium oxalate monohydrates are reviewed by a pathologist.
- **Result Reporting:** Qualitative report of crystals seen – rare, few, moderate, many.
- **Special Considerations:** Artifactual crystals can form in vitro and crystals formed in vivo can dissolve. A fresh sample sent overnight on cold packs will yield the best results.



Urinary crystal identification - Calcium carbonate crystals



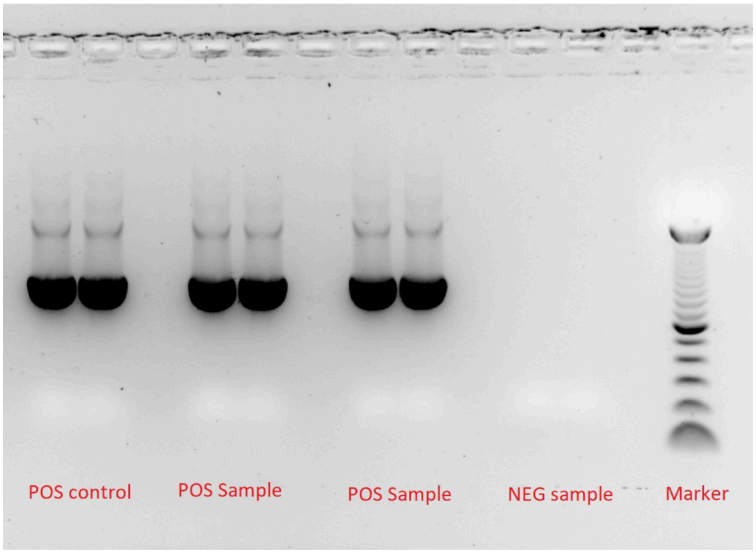
Urinary crystal identification – Cysteine crystals

Urinary crystal identification – Struvite crystals (feline)

Molecular Diagnostics

Gel PCR

- **Diagnostic Use:** Detection of target nucleic acids via polymerase chain reaction. Sometimes referred to as conventional or traditional PCR.
- **Testing Procedure:** Extracted and purified nucleic acids are amplified by PCR and separated by molecular weight on an agarose or polyacrylamide gel matrix subjected to an electrical pulse field.
- **Result Reporting:** Detected/Not Detected
- **Special Considerations:** Same as other PCR assays.



PCR Gel Example

Polymerase Chain Reaction (PCR)

- **Diagnostic Use:** Detection of pathogens' nucleic acids by amplifying them with target-specific reagents.
- **Testing Procedure:** Nucleic acids are extracted and purified from the sample. The product is subjected to alternating cycles of heating (denaturation of the DNA double helix), cooling (attachment of the target-specific primers to the single DNA chains), and gradient temperature increase (synthesis of new DNA chains). The result is the exponential amplification of the target nucleic acid for which the primers were designed. Typically used as a gel PCR or real-time PCR.
- **Result Reporting:** Detected/Not Detected or Positive/Negative depending on the assay.
- **Special Considerations:** PCR can be sensitive

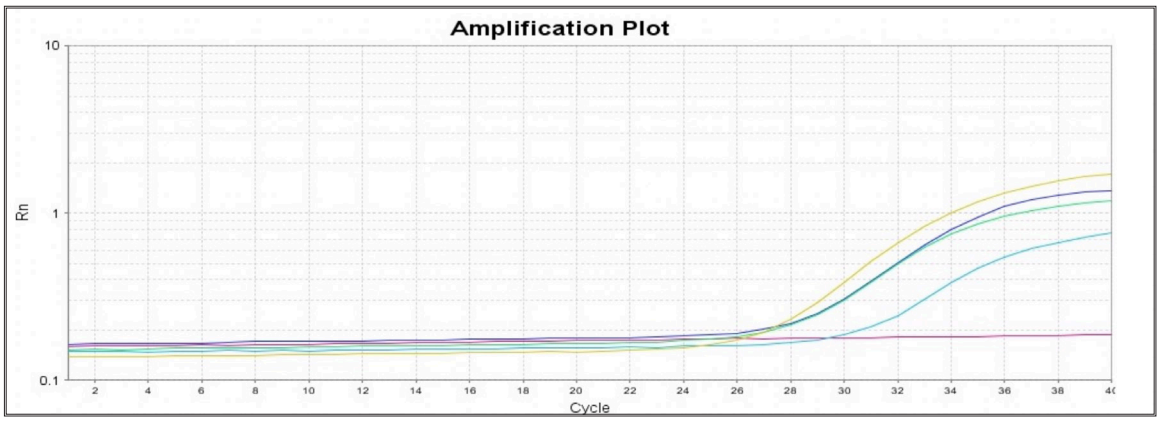
Automated high throughput nucleic acid purification/extraction machine

to substances that inhibit the function of the enzymes used in the amplification procedure. Examples include heparin, gel swabs, and the phenol used in some wooden swab production. Some assay-specific sample types apply. Fresh tissues or whole EDTA blood are generally required. Please see the TVMDL website for more information.

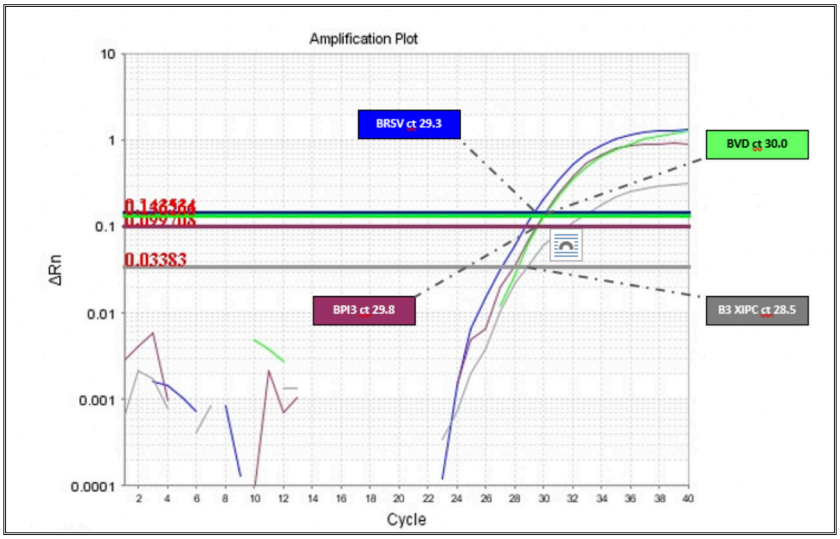
PCR Thermocyclers

PCR Multiplex

- **Diagnostic Use:** Simultaneous detection of different targets in a single PCR reaction.
- **Testing Procedure:** Nucleic acids are purified and amplified using either traditional PCR or real-time PCR. May or may not include reverse transcription. Multiple primers/probes sets are included in a single reaction, each set specific for a particular target, each probe is labeled with a different dye. Distinction of each target is visualized by specific migration patterns through gel by Gel PCR or by using unique fluorescent probes with real-time PCR. In real-time PCR, the machine is able to distinguish between different fluorescent dyes in different probes, each of which is designed to be specific to the different tested targets.
- **Result Reporting:** Reported using Gel PCR or real-time PCR, generally as Detected or Not Detected. May or may not include a Ct value.
- **Special Considerations:** Same as other PCR assays.



PCR Multiplex Amplification Plot – note the different colors/lines for different targets



PCR Multiplex Amplification Plot – Diagnostic specimen positive for BPI3, BVD, and BRSV using the B3 multiplex assay. XIPC is the internal control.

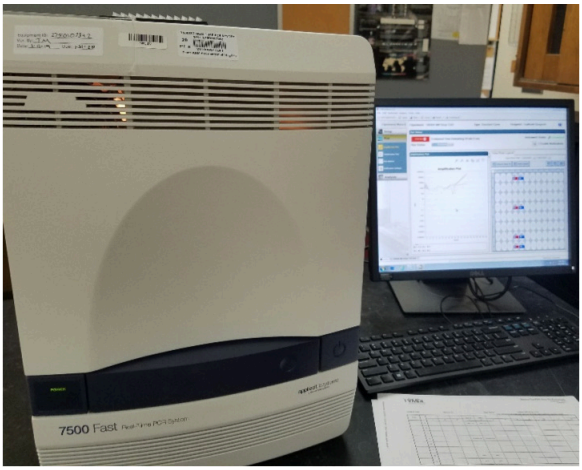
Quantitative PCR (qPCR)

[also known as real time PCR (rtPCR)]

See "Real time PCR" below

Real Time PCR (rtPCR)

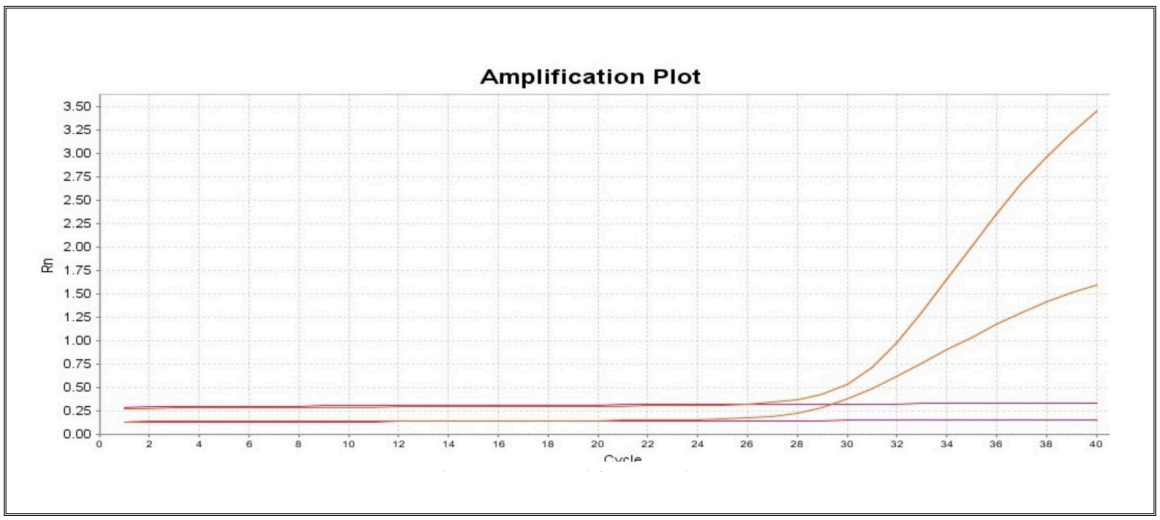
[also known as quantitative PCR (qPCR)]



Real time PCR system

- **Diagnostic Use**: Detection of target nucleic acids via polymerase chain reaction.
- **Testing Procedure:** Extracted and purified nucleic acids are subjected to PCR in the presence of target-specific primers with the addition of probes labeled with a fluorescent (inactive in intact probes). Probes are specific to the amplified DNA and are hydrolyzed during the amplification process which activates the fluorescent dye. The change in fluorescence emissions is measured after each cycle by the machine and displayed visually by a cycle number vs change in fluorescence levels. A threshold cycle (Ct) value is produced by reporting the number of cycles it takes for the fluorescent emission levels to cross a threshold line. This threshold line is specified for each target and is the value where the fluorescence exceeds background/non-specific levels. The Ct value is inversely proportional to the amount of target nucleic acids. Lower Ct values indicate larger amounts of initial target and can be useful in estimating the overall pathogen load in the sample.
- **Result Reporting:** Results are generally reported as Detected/Not Detected followed by the Ct value. Occasionally may be reported as Positive/Negative and may or may not include a Ct value.

- **Special Considerations:** Same as other PCR techniques. Generally considered more specific than traditional PCR due to the inclusion of target-specific probe/s (aka “third” primer/s) alongside the amplification primers.



Real time PCR amplification plot

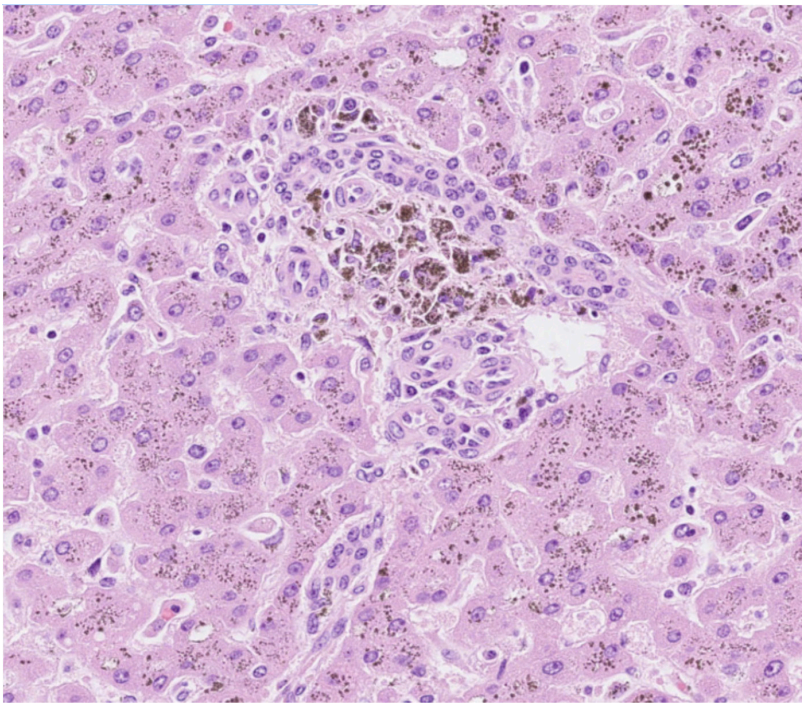
Reverse Transcription PCR

- **Diagnostic Use:** Used to amplify target RNA.
- **Testing Procedure:** RNA is extracted and purified from a sample and converted to its complementary DNA (cDNA) using the enzyme reverse transcriptase. Amplification of the cDNA proceeds using traditional PCR or real-time PCR.
- **Result Reporting:** Reported using Gel PCR or real-time PCR, generally Detected or Not Detected.
- **Special Considerations:** In addition to the sample requirements for most PCR assays, RNA is particularly sensitive to degradation until it can be purified from the sample. Fresh, chilled samples are ideal.

Necropsy and Histopathology

Biopsy

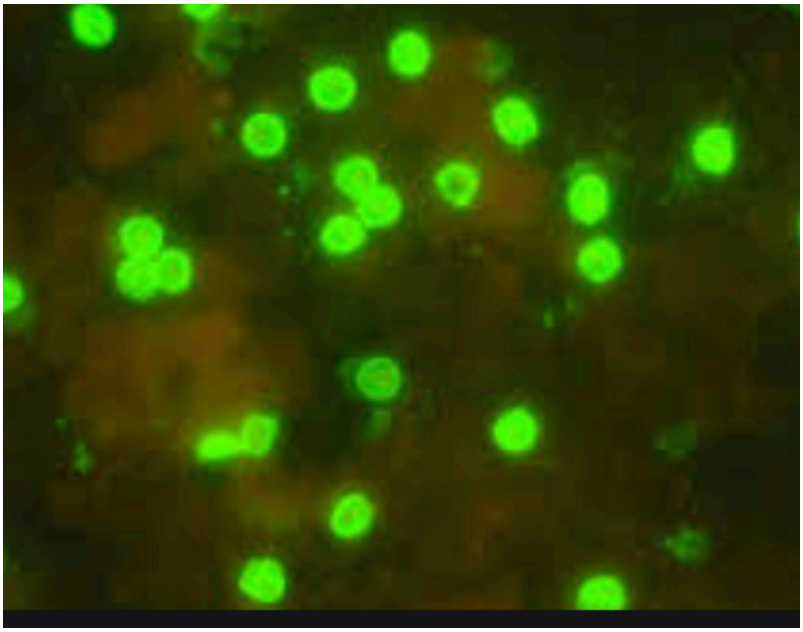
- **Diagnostic Use:** The characterization of disease process in living animals with the goal of determining the etiology.
- **Testing Procedure:** A surgical procedure where a tissue sample is removed from a living animal. Different types are called variously – needle biopsy, punch biopsy, incisional biopsy, or excisional biopsy. In another sense whole organ removal (splenectomy) or limb amputation would be extreme examples of a biopsy.
- **Result Reporting:** Report of pathologist's findings, interpretations, and comments
- **Special Considerations:** Large unfixed samples and samples with bone require additional processing time for either complete fixation and/or decalcification.



Biopsy

Direct Fluorescent Antibody Test (FA)

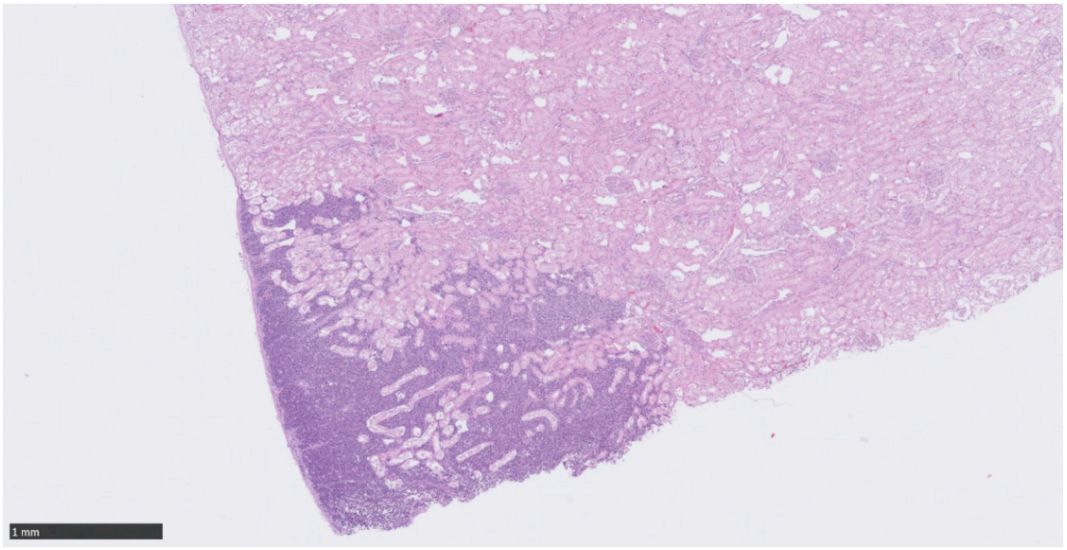
- **Diagnostic Use:** Specific identification of microorganisms in tissue specimens.
- **Testing Procedure:** Antibodies to specific microorganisms and tagged with a fluorescent marker are utilized to identify the underlying etiology in tissues.
- **Result Reporting:** Report of findings as either positive or negative for a specific agent.
- **Special Considerations:** The test is run on fresh tissue.



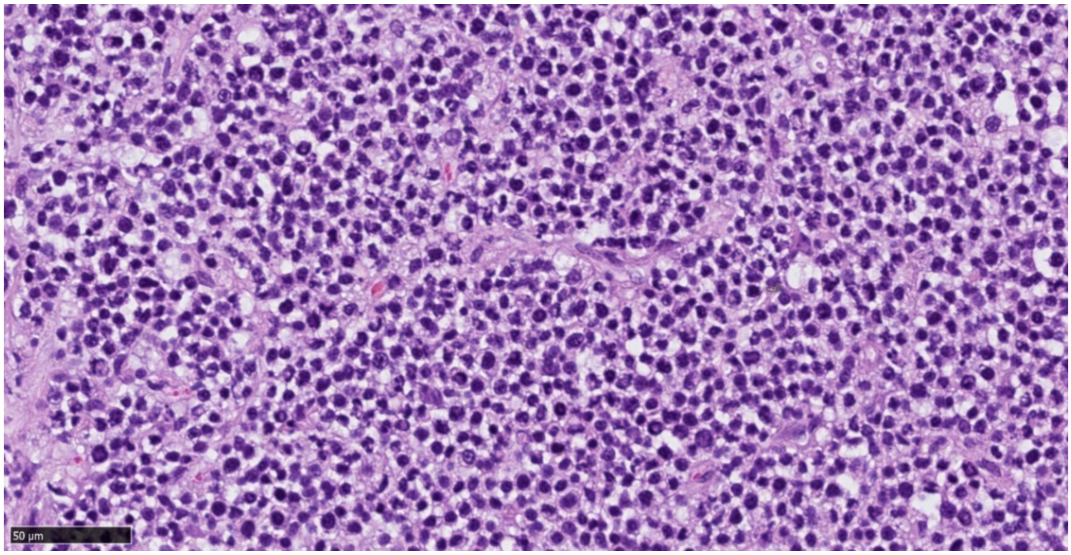
Direct Fluorescent Antibody Test (FA)

Histopathology

- **Diagnostic Use:** Determination of any microscopic lesions that with interpretation can identify an underlying etiology to the disease process.
- **Testing Procedure:** The microscopic examination of tissues collected either by biopsy or necropsy. Tissues typically fixed in 10% neutral buffered formalin and processed to allow very thin sections prepared for microscopic examination by a pathologist.
- **Result Reporting:** Report of pathologist's findings, interpretations, and comments
- **Special Considerations:** Large unfixed samples and samples with bone require additional processing time for either complete fixation and/or decalcification.



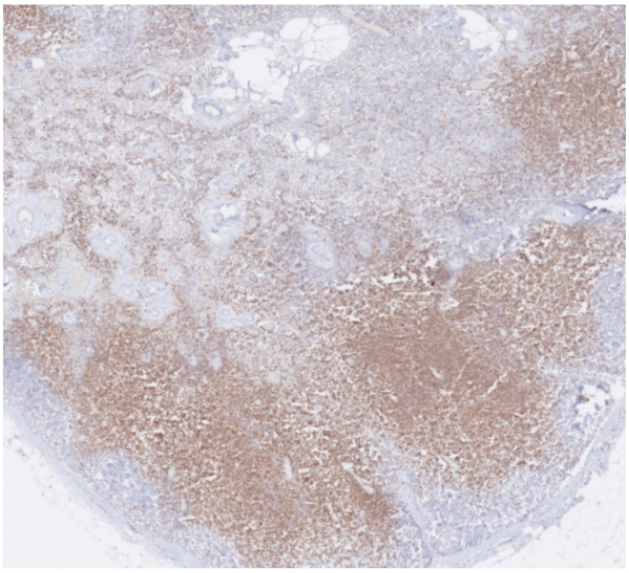
Histopathology – Kidney of goat with Lymphoma, low magnification



Histopathology – Mesentery from same goat, high magnification

Immunohistochemistry (IHC)

- **Diagnostic Use:** Specific identification of either infectious organism or tumor type in fixed tissue samples.
- **Testing Procedure:** represents a highly specific and sensitive type of special stain. It utilizes antigen-antibody reactions to identify cell/tissue components, prions, or microorganisms whether bacterial, fungal, parasitic, or viral. IHC also uses formalin-fixed tissues and similar processing to produce the thin sections. However, it applies a different staining protocol where antibodies directed at a specific target are applied to the tissue section and then secondarily exposed to a second antibody that contains a colored product that then is visible under the microscope.
- **Result Reporting:** Report of pathologist's findings, interpretations, and comments
- **Special Considerations:** The test can be run on formalin fixed and embedded tissue submitted to the lab. Some tests are performed in-house while others are on a referral basis. IHC typically requires an additional 5-10 days for reporting.



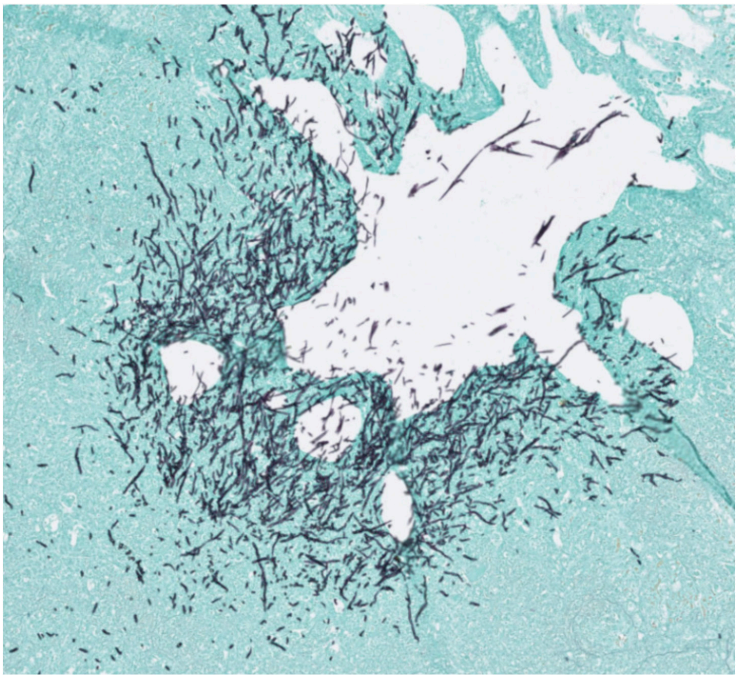
Lymph Node IHC – CD3 for Lymphoma

Necropsy

- **Diagnostic Use:** Examination of an animal carcass to identify a disease process or cause of death.
- **Testing Procedure:** sometimes referred to as autopsy or postmortem exam is a procedure where a dissection is performed on an animal carcass. Depending on the clinical picture, these may be limited to a particular organ system or body cavity or may be complete meaning all major organ systems are examined. A necropsy is usually done to examine the organs and take samples for the determination of a cause of death.
- **Result Reporting:** Report of pathologist's findings, interpretations, and comments
- **Special Considerations:** Postmortem interval should be minimal to guarantee best possible results. Carcasses in an advanced state of decomposition are poor specimens.

Special Staining

- **Diagnostic Use:** Chemical staining procedures applied to tissue section in order to identify various cellular products in tissue sections examined microscopically
- **Testing Procedure:** Before microscopic examination can be performed, the sections prepared above must be stained for visualization. The routine stain for this is an H&E (hematoxylin and eosin) stain. Tissues appear in various shades of pink and blue. In some cases, the pathologist may request other chemical stains that help in the identification of certain components in the tissues. For example, there are chemical stains for iron, copper, bile, fibrous tissue, fibrin, and striations in muscle fibers.
- **Result Reporting:** Report of pathologist's findings, interpretations, and comments
- **Special Considerations:** Staining is performed on formalin-fixed tissue samples

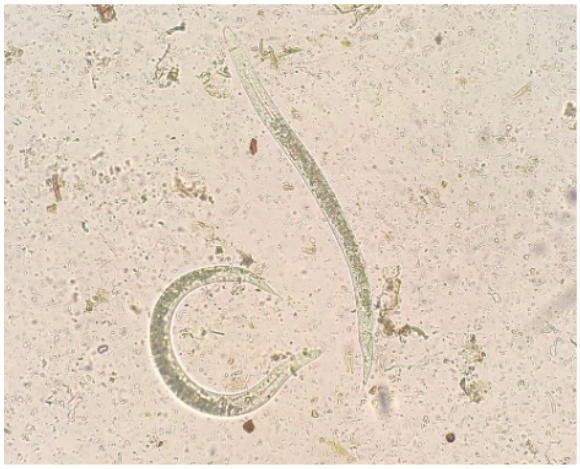


Special Staining

Parasitology

Baermann Test

- **Diagnostic Use:** For the detection of lungworm and other parasitic larvae in fresh feces.
- **Testing Procedure:** A fecal bag (feces wrapped with gauze) is placed in a funnel affixed with tubing and a vial. The funnel is filled with warm water and set for several hours. If larvae is present, they wiggle out of the fecal packet and sink to the bottom of the vial. The sediment in the vial is examined under the microscope by a highly trained parasitology technician for the presence of larvae.
- **Result Reporting:** Species of larvae seen (i.e. *Dictyocaulus viviparous*) or no larvae observed.
- **Special Considerations:** None



Dictyocaulus filaria larvae recovered from goat feces via the Baermann test.

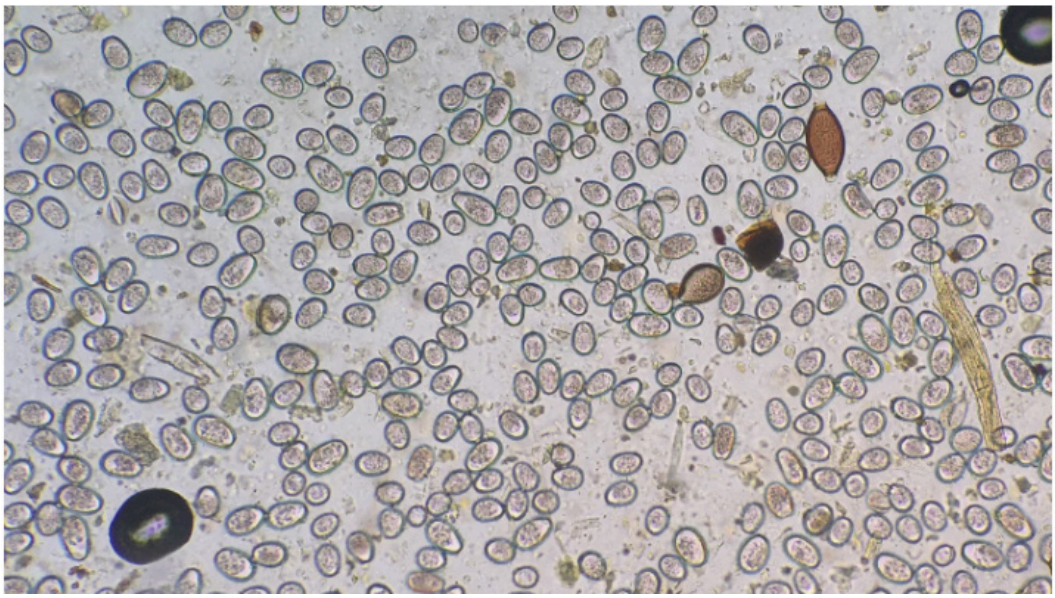
Copraculture

- **Diagnostic Use:** For the differentiation of Trichostrongyle type eggs by 3rd stage larvae identification.
- **Testing Procedure:** Known positive fecal samples (minimum 50 Trichostrongyle type egg per gram count) are kept in a moist and humid environment for 2-3 weeks to facilitate egg hatching and larval growth. After 2-3 weeks, the fecal bag (feces wrapped with gauze) is placed in a funnel affixed with tubing and a centrifuge vial at the end. The funnel is filled with warm water and set for several hours. Larvae wiggle out of the fecal packet and sink to the bottom of the centrifuge vial. The sediment or bottom of the centrifuge vial is examined under the microscope for the presence of 3rd stage larvae.
- **Result Reporting:** Specific Trichostrongyle type parasite and the percentage seen (*Haemonchus contortus* 90%, *Ostertagia ostertagi* 10%).
- **Special Considerations:** This is a referral test. A McMasters egg/gram count is recommended in conjunction with this test. A minimum 50 Trichostrongyle type egg per gram count is necessary for enough larvae recovery.

Fecal Flotation (Qualitative)

- **Diagnostic Use:** For the detection of fecal parasite eggs, oocysts, and cysts.
- **Testing Procedure**: 1-2 grams of fecal material is mixed with appropriate flotation solution. Fecal solution is strained, poured into a centrifuge tube, coverslipped, and centrifuged. After centrifugation, the coverslip is removed and placed on a microscope slide. The entire coverslip is examined on a compound microscope by a highly trained parasitology technician.
- **Result Reporting:** Parasite(s) identified (if any) and approximate quantity (rare, few, moderate, or many).
- **Special Considerations:** This test is ideal for fecal parasite detection in small animals, reptiles,

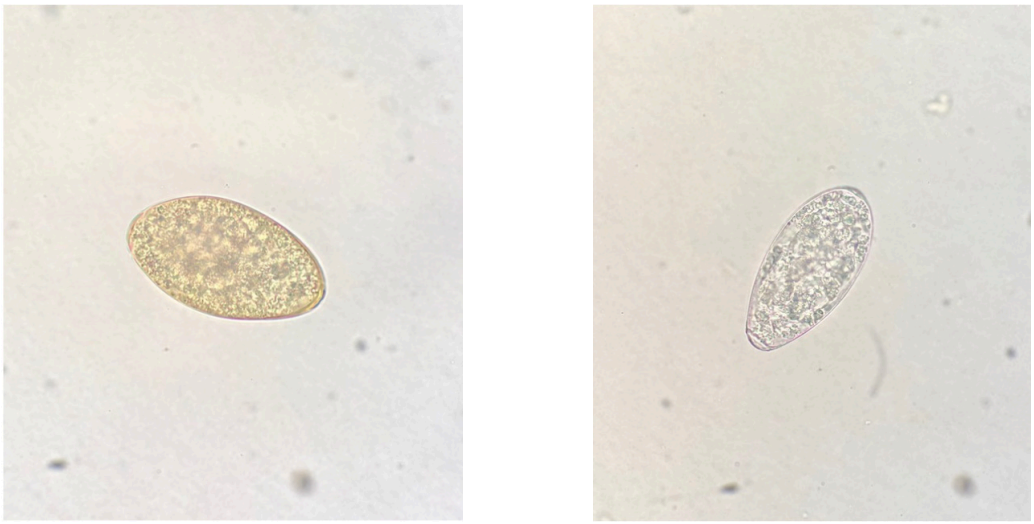
and birds. Low numbers of Cryptosporidium and *Giardia* spp. may not be detected with this test. A McMasters eggs/gram count is recommended in equids, camelids, small ruminants, and calves. A Wisconsin eggs/gram counts is recommended for adult cattle.



Numerous *Eimeria* spp. oocysts and a *Trichuris* sp. egg recovered from calf feces.

Fecal Sedimentation

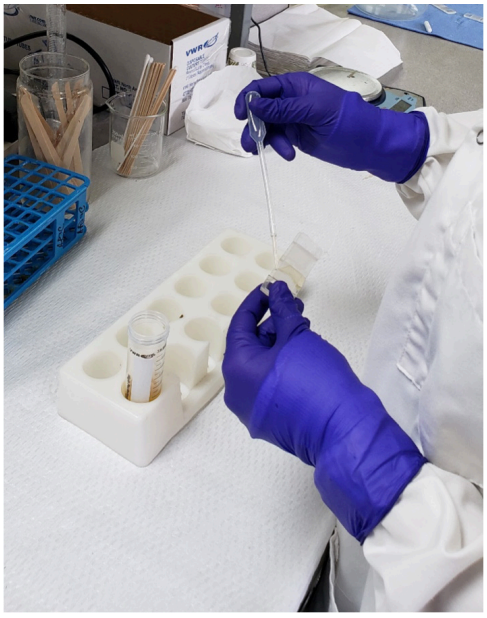
- **Diagnostic Use:** For the detection of fluke eggs.
- **Testing Procedure:** Feces is mixed with water and strained through two layered screens. The top screen catches large debris and is discarded. The bottom screen catches smaller debris and fluke eggs. The material from the bottom screen is washed into a petri dish and examined by a highly trained technician under a dissecting microscope for fluke eggs.
- **Result Reporting**: Fluke eggs seen, if any (i.e. *Fasciola hepatic, Paramphistomum* sp.).
- **Special Considerations:** Infected animals will not pass eggs until they have been infected for several months. If flukes are suspected, repeat testing may be necessary.



Fasciola hepatica (left) and *Paramphistomum* sp. (right) fluke eggs recovered from bovine feces.

McMasters Eggs/Gram Count

- **Diagnostic Use**: For quantitative egg count detection for parasites in feces.
- **Testing Procedure:** 2 grams of feces is mixed with 28 mL of saturated salt solution. A portion of the well-mixed fecal solution is loaded into a McMasters counting chamber. Using a compound microscope, the number of parasite eggs and/or oocysts seen within the gridlines of the McMaster counting chamber is counted and multiplied by 50.
- **Result Reporting**: Parasite eggs or oocysts seen (if detected) with egg per gram count (i.e. Trichostrongyle type ova 100 eggs/gram, *Eimeri*a sp. 500 oocysts/gram)
- **Special Considerations:** This test can only detect fecal parasites when burden is 50 eggs per gram or higher. Ideal test for horses, camelids, small ruminants, calves, and animals suspected to have higher egg counts (>50 eggs per gram).



McMasters egg/gram count – loading sample into McMasters counting chamber

Parasite Examination – Mite or Helminth

- **Diagnostic Use:** For the identification of various parasites (helminths, ticks, mites, etc.).
- **Testing Procedure:** Gross and microscopic evaluation of the parasite. Examine external and internal features to help aid in identification. For helminths, a glycerol solution may be used to 'soften' the exterior, allowing for viewing of internal organs (i.e. spicules, eggs).
- **Result Reporting:** Identification of parasite, usually to genus or species level, or identified as non-parasitic (i.e. man-made fiber, plant fiber, non-parasitic worm).
- **Special Considerations:** Submit whole specimen in 70% alcohol for preservation. 10% formalin can be used, but is not recommended for helminths.

Male and female *Amblyomma maculatum* ticks

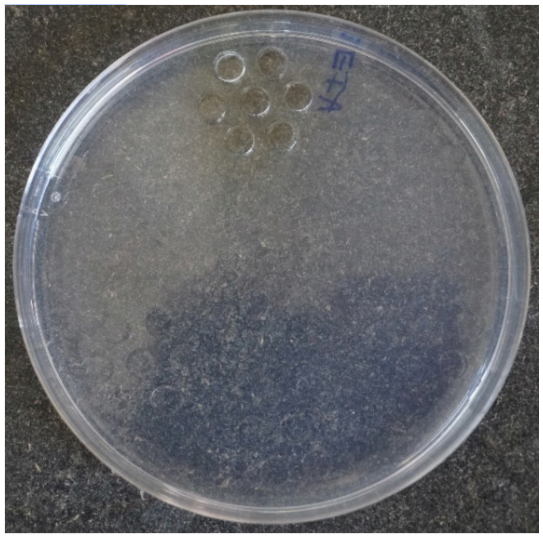
Wisconsin Eggs/Gram Count

- **Diagnostic Use:** For quantitative egg count detection for parasites in feces.
- **Testing Procedure:** 5 grams of fecal material is mixed with water, strained, and centrifuged to help aid in the removal of debris. The supernatant is discarded and the pellet/sedimentation is mixed with saturated sugar solution, coverslipped, and centrifuged again. Saturated sugar solution has a high specific gravity and allows for parasite eggs and oocysts to float more readily. The entire coverslip is examined under a microscope and any eggs or oocysts detected are counted.
- **Result Reporting:** Parasite eggs or oocysts seen (if detected) with egg per gram count (i.e. Trichostrongyle type ova 5.2 eggs/gram, Eimeria sp. 22.6 oocysts/gram).
- **Special Considerations:** Ideal test for adult cattle and other large ruminants. Detects very low numbers of parasite eggs (as low as 0.2 eggs per gram).

Wisconsin eggs/gram – mounting coverslip on sample before second centrifuge step

Serology

Agar-Gel Immunodiffusion (AGID)

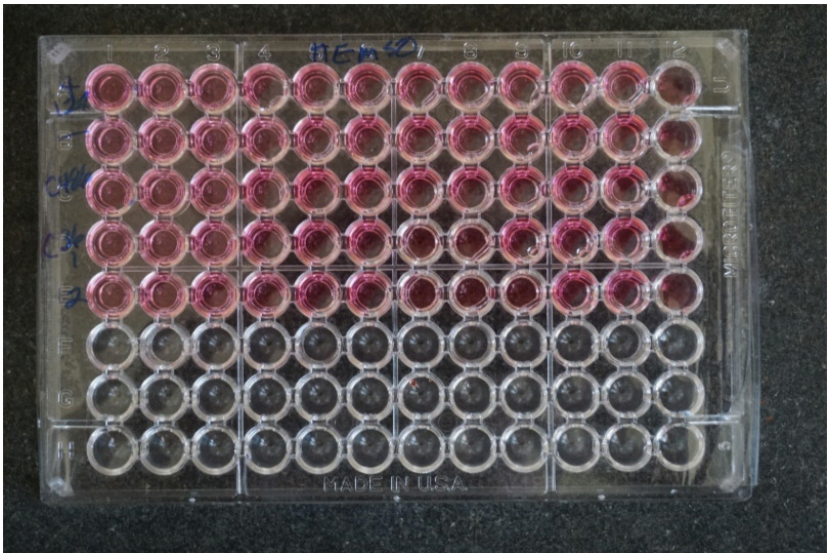


AGID plate

- **Diagnostic Use:** The AGID test is utilized to detect antibodies to the targeted disease organisms, usually from a serum sample.
- **Testing Procedure:** Sample wells are cut into an agar plate in a pattern equidistant from a central well which contains antigen from the targeted organism. The outer sample wells contain serum from the animals being tested and known positive antibody samples in an alternating pattern. A line of precipitation between the central and test well indicates a positive reaction.
- **Result Reporting:** This is a non-automated test so there is some subjectivity involved with result interpretation. Results are reported as negative or positive.
- **Special Considerations:** AGID is generally considered to be more specific and less sensitive than an ELISA test, so is typically used as a diagnostic test for a clinically ill animal vs. herd screening test, or as a confirmatory test for a (+) ELISA result.

Agglutination Test

- **Diagnostic Use:** Agglutination tests detect antibodies to the targeted disease organisms, usually from a serum sample.
- **Testing Procedure:** Serum samples are exposed to antigenic material from the targeted disease organism, and if antibodies to the organism are present in the sample, clumping or agglutination reactions will occur.
- **Result Reporting:** This is a non-automated test so there is some subjectivity involved with result interpretation. Results are reported as positive or negative.
- **Special Considerations:** None



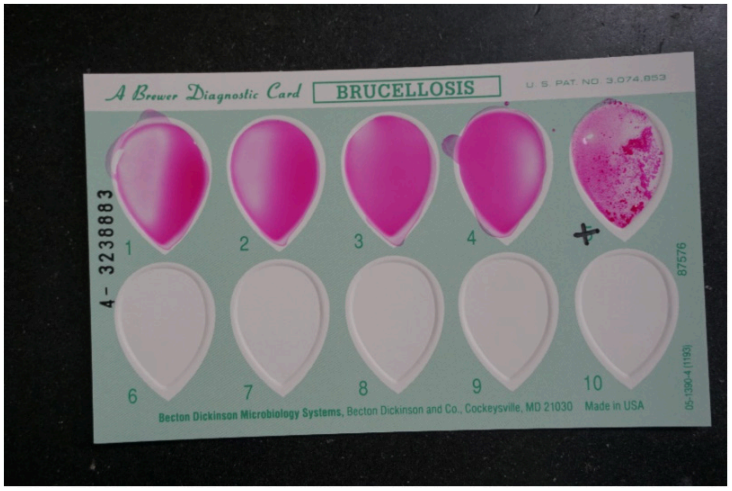
Agglutination test

Antigen Capture ELISA (Ag ELISA)

- **Diagnostic Use:** The Antigen Capture ELISA test is used to detect antigenic or toxic material for the targeted organism in a sample specimen.
- **Testing Procedure:** Antigens from the sample are attached to a surface, and a matching antibody is applied over the surface to create binding of the antigen and antibody. The antibody is linked to an enzyme, and the enzyme's substrate is added in the final testing step. The reaction produces a detectable signal, most commonly a color change.
- **Result Reporting:** Testing is typically performed using a 96 well plate that is read by an automated plate reader. Internal calculations of the machine, which depend on the amount of light passing through each sample well, provide results that are reported as positive, suspicious, or negative.
- **Special Considerations:** ELISA tests typically have high sensitivity (i.e. detect positive animals but may have some false positive samples) making them a good screening test. Positive or suspect results on regulatory tests are sent to either a state or federal lab for confirmatory testing.

Card Agglutination

- **Diagnostic Use:** Agglutination tests detect antibodies to the targeted disease organisms, usually from a serum sample. See Agglutination tests.
- **Testing Procedure:** Sample and test antigen are combined on a special card which enables a reaction to occur on a positive sample.
- **Result Reporting:** This is a non-automated test so there is some subjectivity involved with result interpretation. As the only bovine card test is a regulatory test, only negative results are reported. Non-negative results are sent to NVSL for confirmatory testing.
- **Special Considerations:** None



Card Agglutination

Complement Fixation (CF)

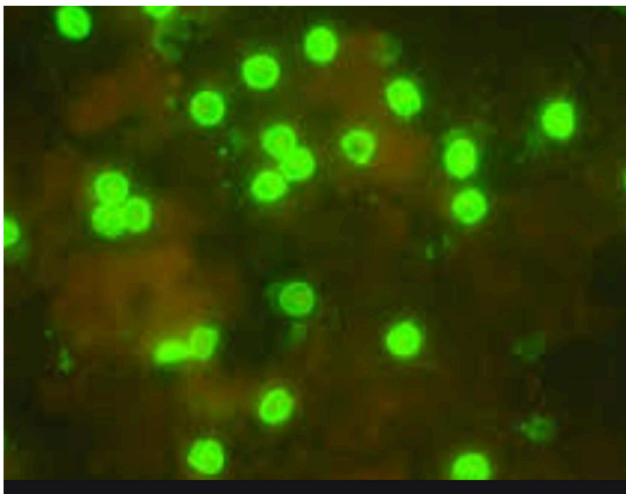
- **Diagnostic Use:** The Complement Fixation test detects antibodies for the targeted organism in a serum sample.
- **Testing Procedure:** Sample and known antigen are mixed with complement, sheep red blood cells (SRBCs) and an antibody against SRBCs, and then the degree of lysis of the SRBCs is measured. If antibodies to the organism are present in the serum, antigen-antibody complexes form which bind the complement and inhibit the lysis of the SRBCs, indicating a positive test.
- **Result Reporting:** This is a non-automated test so there is some subjectivity involved with result interpretation. The complement fixation test is reported as negative or as positive at an endpoint of increasing dilutions of the sample (i.e. titer).
- **Special Considerations:** No bovine tests at TVMDL utilize this testing methodology. Samples forwarded to NVSL for various requested tests are tested by this method.

Competitive (Inhibitive) ELISA (cELISA)

- **Diagnostic Use:** This test detects antibodies for the targeted organism in a serum sample.
- **Testing Procedure:** The competitive ELISA process sets up a competition for antibody between antigen present in the sample and added antigen so that color change is inversely proportional to amount of antibody present in the sample.
- **Result Reporting:** Test is typically performed using a 96 well plate that is read by an automated plate reader. Internal calculations of the machine, which depend on the amount of light passing through each sample well, provide results that are reported as positive, suspicious, or negative.
- **Special Considerations:** ELISA tests typically have high sensitivity (i.e. detect positive animals but may have some false positive samples) making them a good screening test.

Direct Fluorescent Antibody Test (FA)

- **Diagnostic Use:** The fluorescent antibody test detects antigenic material from the targeted organism in a fresh or fixed tissue sample.
- **Testing Procedure:** Fluorescent chemicals are attached to areas of an antibody, and if antigenic material is present in the sample, it will attach to the to the antibody and fluorescence will occur. This fluorescence is detected with the aid of dark field illumination under a microscope.
- **Result Reporting:** This is a non-automated test so there is some subjectivity involved with result interpretation. Results are reported as positive or negative.
- **Special Considerations:** This testing methodology is performed in the Bacteriology section for identification of some clostridial organisms.



Direct Fluorescent Antibody Test (FA)

Enzyme Linked Immunosorbent Assay (ELISA)

- **Diagnostic Use:** The ELISA test detects antibodies for the targeted organism in a serum sample.
- **Testing Procedure:** The ELISA process sets up antigen-antibody reactions linked to an enzyme.
- **Result Reporting:** Testing is typically performed using a 96 well plate that is read by an automated plate reader. Internal calculations of the machine, which depend on the amount of light passing through each sample well, provide results that are reported as positive, suspicious, or negative.
- **Special Considerations:** ELISA tests typically have high sensitivity (i.e. detect positive animals but may have some false positive samples), making them a good screening test.

Immunoperoxidase Test (IPT)

- **Diagnostic Use:** The IPT test is used to detect antibodies for the targeted organism in a serum sample.
- **Testing Procedure:** The IPT test method enables antigen and antibody reactions to occur, then uses the immunoperoxidase enzyme to cause detectable color changes in the reaction.
- **Result Reporting:** The results are reported as positive or negative.
- **Special Considerations:** No testing at TVMDL utilizes this testing methodology. Samples forwarded to NVSL for Malignant Catarral Fever are tested by this method.

Indirect Florescent Antibody Test (IFA)

- **Diagnostic Use:** The IFA test utilizes specially prepared antigen containing slides to detect antibodies for the targeted organism in a serum sample.
- **Testing Procedure:** This test also depends on the development of antigen-antibody reactions and utilizes a chemical to identify the reaction by fluorescing, which is detected with the aid of dark field illumination under a microscope.
- **Result Reporting:** This is a non-automated test so there is some subjectivity involved with result interpretation. Results are reported as negative or as a titer at the last dilution at which antibodies were detected.
- **Special Considerations:** None

Indirect Fluorescent Antibody Test (IFA)

Kinetic ELISA (k-ELISA)

- **Diagnostic Use:** The k-ELISA test is used to detect antibodies for the targeted organism in a serum sample.
- **Testing Procedure:** The k-ELISA method is a specialized form of ELISA testing which is better able to quantify the amount of antigen present in a sample compared to the regular ELISA test. This method measures the level of antigen-antibody reactions that take place over time.
- **Result Reporting:** Results are reported as negative or positive (>2 or >1000)
- **Special Considerations:** No testing at TVMDL utilizes this testing methodology. Samples forwarded to Cornell University Animal Health Diagnostic Center for Bovine Herpesvirus Type 1 (IBR) are tested by this method.

Latex Agglutination (LA)

- **Diagnostic Use:** The LA test is able to detect antigens or antibodies, depending on which is targeted, in body fluids.
- **Testing Procedure:** Latex beads are coated with either antigens or antibodies, and antigen-antibody reactions are allowed to occur on the beads.
- **Result Reporting:** Results are reported as positive or negative.
- **Special Considerations:** No bovine testing at TVMDL utilizes this testing methodology.

Microscopic Agglutination Test

- **Diagnostic Use**: The microscopic agglutination test (MAT) detects antibodies to targeted organisms in a serum sample and utilizes a chemical to identify the reaction by fluorescing, which is detected with the aid of a dark field illumination under a microscope. This test is most commonly used to test for antibodies to Leptospira organisms.
- **Testing Procedure:** The MAT depends on subjecting wells of live organisms to diluted samples of serum from the subject animal. Antibodies present in the serum sample cause the organisms to die, and the degree of organism death is used to determine a positive or negative result at each dilution.
- **Result Reporting:** This is a non-automated test so there is some subjectivity involved with result interpretation. Samples are initially screened at the starting dilution (i.e. 1:100, except for export cases requesting 1:400). In the case of a positive result at the first dilution, the sample is subjected to increasing dilutions. Results are reported as negative or as a positive titer at the last dilution at which antibodies were detected.
- S**pecial Considerations:** There is much cross-reactivity between the various serovars.

Plate Agglutination (Standard Plate Test – SPT)

- **Diagnostic Use:** The plate agglutination test detects antibodies for the targeted organism in a serum sample. See Agglutination tests.
- **Testing Procedure:** The plate agglutination test relies on the precipitation of antigen-antibody reactions on a special plate at various dilutions.
- **Result Reporting:** This is a non-automated test so there is some subjectivity involved with result interpretation. As the only bovine SPT is a regulatory test, only negative results are reported. Non-negative results are sent to NSL for confirmatory testing.

- **Special Considerations:** None

Radial Immunodiffusion (RID)

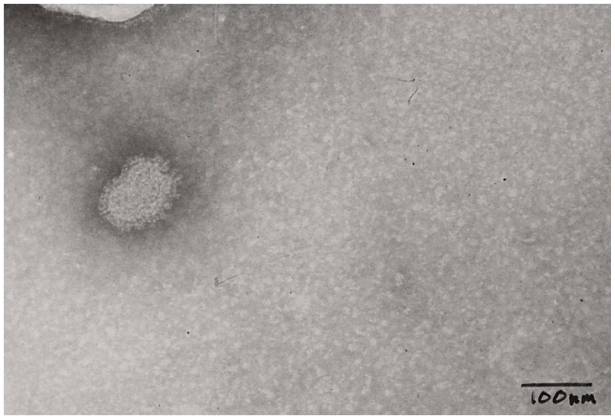
- **Diagnostic Use:** The RID test detects targeted antigens in a sample.
- **Testing Procedure:** The sample being testing is placed in a central well on an agar plate, and diffusion is allowed to occur radially to wells containing antibody for the targeted organism. Precipitation of the antigen-antibody reactions occurs in a line in the agar plate.
- **Result Reporting:** Results are reported as positive or negative.
- **Special Considerations:** No testing at TVMDL utilizes this testing methodology.

Turbidometric Immunoassay (TIA)

- **Diagnostic Use:** The TIA test detects antibodies for the targeted organism in a serum sample.
- **Testing Procedure:** The TIA test measures the level of turbidity that occurs when antigen-antibody reactions occur in a test sample.
- **Result Reporting:** Results are reported within a numerical range.
- **Special Considerations:** No testing at TVMDL utilizes this testing methodology. Samples forwarded to Cornell University Animal Health Diagnostic Center for Immunoglobulin G (a measure of passive transfer) are tested by this method.

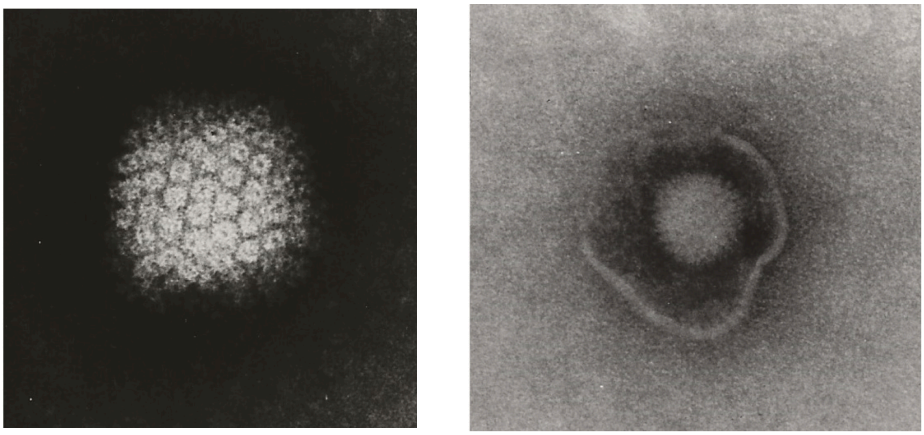
Virology

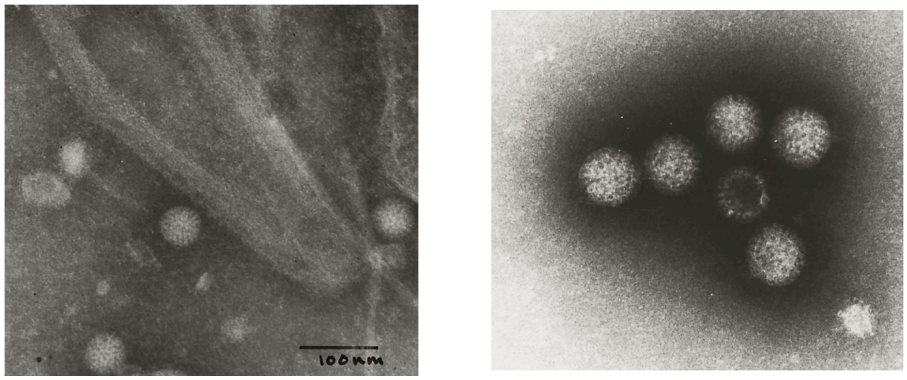
Electron Microscopy (EM)



Coronavirus

- **Diagnostic Use:** (Transmission) Electron microscopy is used to look for viruses in a sample.
- **Testing Procedure:** Samples are prepared as crude sample homogenates, pelleted by ultracentrifugation (approx.. 30,000 x *g*), then mixed with a heavy metal "stain" which binds to the outside of a particle, creating an electron dense matrix. The transmission electron microscope utilize a beam of accelerated electrons which is blocked by the metal (negative staining) allowing for visualization of tiny objects (e.g. viruses), exosomes, etc.).
- **Result Reporting:** Results are reported as either no viruses were observed (no viruses were observable in the sample prepared) or to the virus family if a virus is identified (e.g. parvovirus, adenovirus, herpesvirus, etc.).
- **Special Considerations:** The detection limit of this method is approximately 106 particles per milliliter. The method identifies viruses to the family level (e.g. parvovirus, adenovirus, herpesvirus, etc.) and not to a species level (e.g. canine adenovirus 2, bovine herpesvirus 4, etc.). The preparations are very crude and is different from thin sectioning electron microscopy.


Herpesvirus

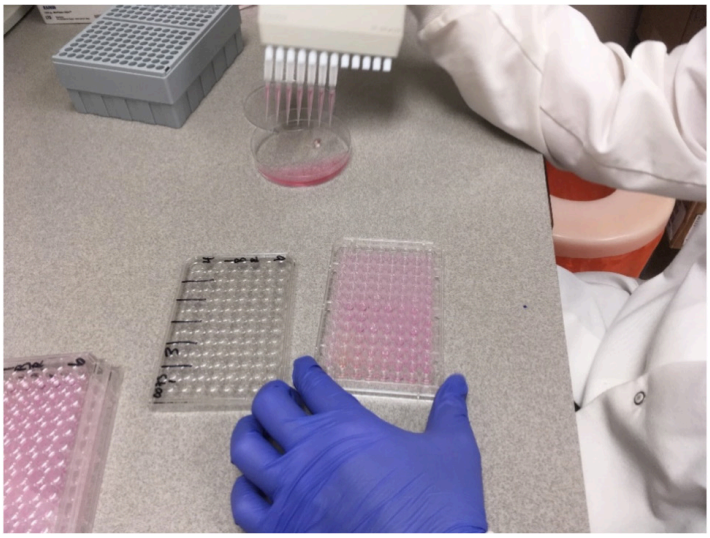


Rotavirus

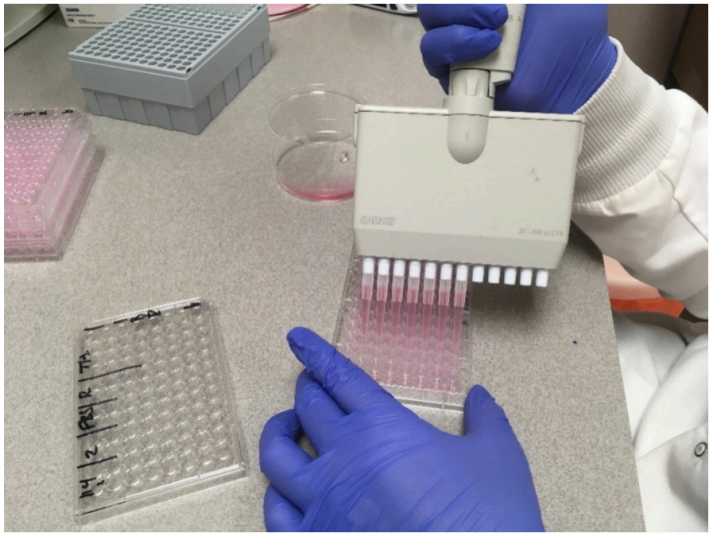
Serum Neutralization (SN)

[also known as the virus neutralization (VN) test]

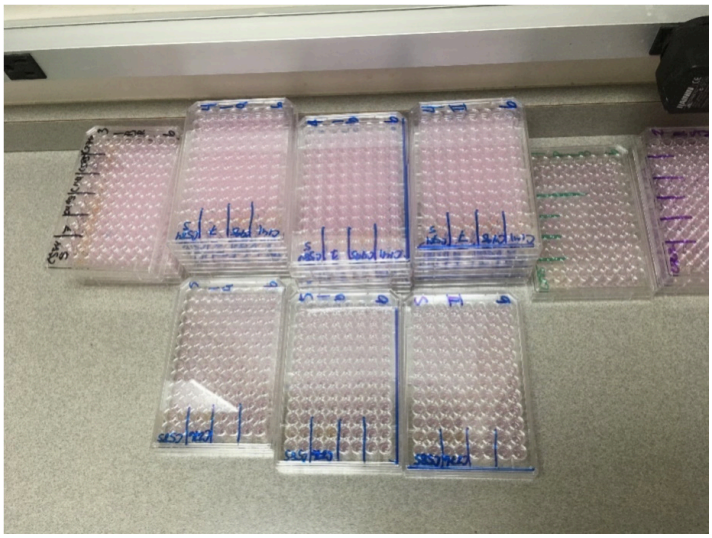
- **Diagnostic Use:** The SN test is utilized to detect neutralizing antibodies in a serum sample to a specific virus.
- **Testing Procedure**: The test sample is heat inactivated, serially diluted, then a measured amount of virus is added to each test sample. The sample/virus mixtures are on test for the required amount of time for the virus to grow in the susceptible cell line (e.g. 3-8 days). If cytopathic effects (CPE) (ie. changes in the cells) are observed then that particular well is considered negative for antibodies to that virus, if CPE is not observed that well is considered positive for antibodies to the virus.
- **Result Reporting:** The results are reported as negative or positive at an endpoint dilution (titer).
- **Special Considerations:** Some samples generate toxicity that kills the cells or can resemble viral cytopathic effects rendering the test unreadable. Plasma is not an appropriate sample for SN/VN testing.



Serum Neutralization – measured amount of virus in diluent being added to the serially diluted serum samples

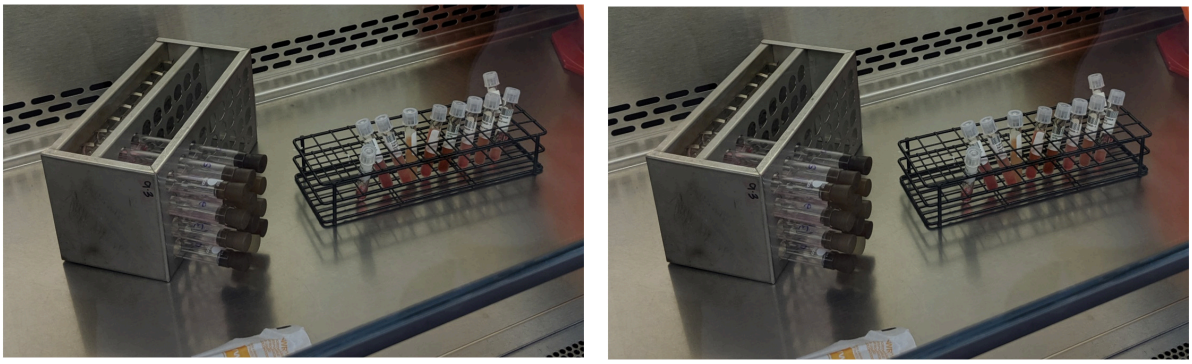


Serum Neutralization – measured amount of virus in diluent being added to the serially diluted serum samples

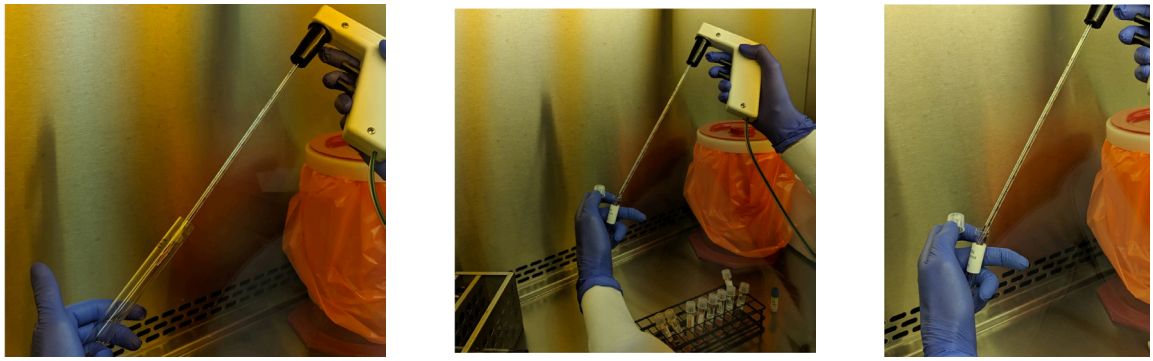


Virus Isolation Via Cell Culture (VI)

- **Diagnostic Use:** The virus isolation test is used to culture or grow viruses from clinical specimens.
- **Testing Procedure:** The sample material is prepared in a viral transport media containing antibiotics then placed in an environment of compatible cells to enhance the growth of viruses.
- **Result Reporting:** Results will be reported as the presence or absence of viral growth and if a virus is grown, the identification of the virus (e.g. bovine viral diarrhea virus, feline calicivirus, etc.).
- **Special Considerations**: Successful virus isolations requires the presence of viable, infectious particles. The success of virus isolation is affected by many factors, such as the condition of the sample received (e.g. refrigerated >48 hrs, abortion, post-mortem >24 hrs, etc.), the immune status of the patient (eg. Immune-compromised, presence of antibodies, etc.), and the phase of infection within the patient (e.g. early, middle, late).



Virus Isolation – Leighton tubes containing cell cultures and samples for inoculation



Virus Isolation – sample inoculation into Leighton tubes

Virus Neutralization (VN)

[also known as the serum neutralization (SN) test]

See “Serum neutralization” above

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Nicholas Monday
Pictures: Kiril Dimitrov

Necropsy and Histopathology Testing
SME: Gayman Helman
Editor: Jessie Monday
Pictures:
- Biopsy – Gayman Helman
- FA – Gayman Helman
- Histopathology – Gayman Helman, Will Sims
- IHC – Gayman Helman
- Necropsy
- Special staining – Gayman Helman

Parasitology Testing
SME: Julie Piccione
Editor: Jessie Monday
Mindy Borst
Pictures:
- Baermann test – Mindy Borst
- Fecal Float – Mindy Borst
- Fecal Sedimentation – Mindy Borst
- McMasters – Dana Burk, Aubrey Waggoner
- Parasite Examination – Mindy Borst
- Wisconsin – Dana Burk, Aubrey Waggoner

Serology Testing
SME: Melanie Landis
Editor/Writer: Guy Sheppard
Pictures:
- All except FA, MALDI – Rusty Raleigh, Stephanie Merrell
- FA – Gayman Helman

Virology Testing
SME: Pam Ferro
Editor: Guy Sheppard
Pictures: Gayla Lovell