TVMDL Texas A&M Veterinary Medical Diagnostic Laboratory

TVMDL Bovine Syndromic Approach to Testing and Diagnostic Plans

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TVMDL Winter Meeting

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Diagnostic Testing & Methodology

What is the purpose of diagnostic testing?

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- To find out what is wrong with our patients

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- ~~To find out what is wrong with our patients~~
- Diagnostic tests are tools of prediction, not explanation
- Results provide evidence to support or refute risk of differential diagnosis

What does that mean?

- Clinician "clinical suspicion of a positive test" before selecting the test has an impact on the interpretation of testing findings
- A positive test result does not always lead to a definitive diagnosis
- A clinical pathology finding within the reference interval is not always normal
- Interpretation of test results and subsequent action all depends on the patient and the history
  - No amount of testing will overcome the need for a good physical exam and understanding of the patient's circumstances (history)

What is the purpose of diagnostic testing?

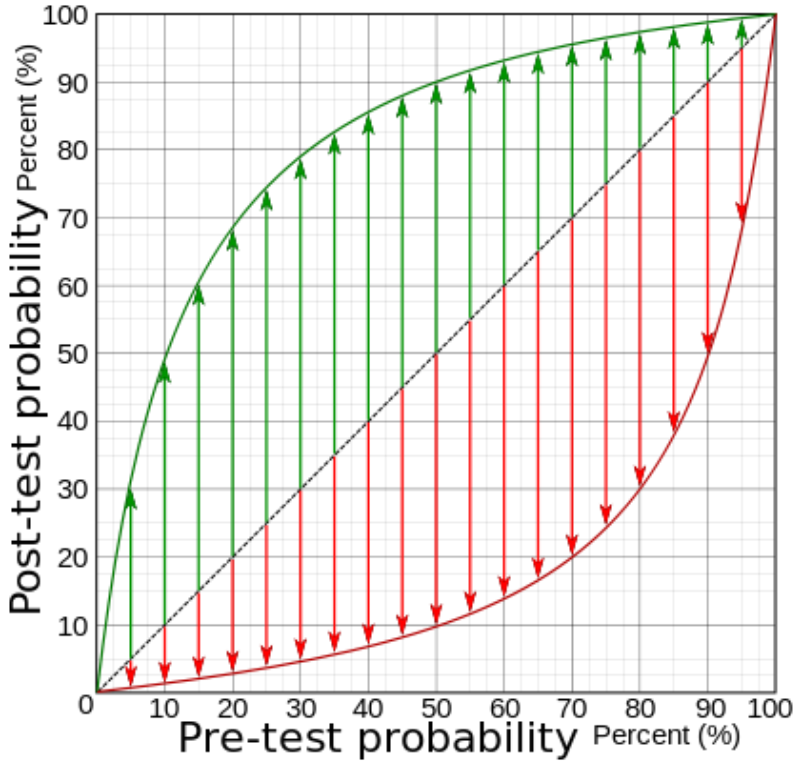
- Diagnosis: to rule in or rule out a specific disease based on pathogen presence, exposure, or physiological effect
- Monitoring: to check response to therapy or the efficacy of preventative, vaccination, or biosecurity programs
- Screening: for genetic diseases, infectious disease carriers, or persistently infected animals
- Research: to investigate specific pathophysiology, disease processes, response to exposure, etc.

What is the purpose of diagnostic testing?

- **Diagnosis**: to rule in or rule out a specific disease based on pathogen presence, exposure, or physiological effect
- Monitoring: to check response to therapy or the efficacy of preventative, vaccination, or biosecurity programs
- Screening: for genetic diseases, infectious disease carriers, or persistently infected animals
- Research: to understand the pathophysiology of a particular disease process

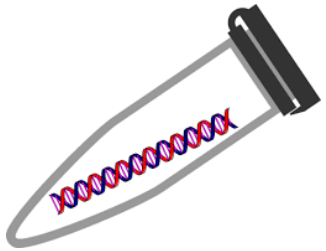
What is the purpose of diagnostic testing

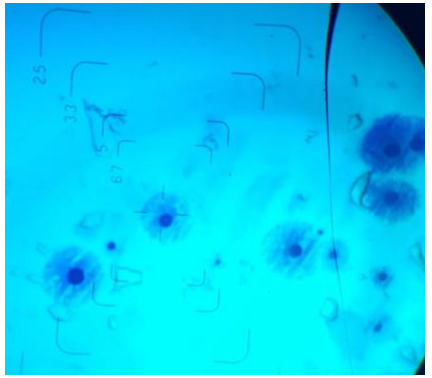
- To gather specific information that closes the gap (amount of uncertainty) between pre-test clinical suspicion and post-test probability of disease
- To inform next steps
  - Treatment & Prognosis
  - Management changes
  - Prevention strategies
  - Additional testing needed

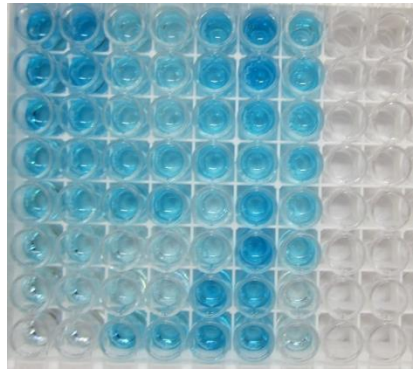


Testing Methods – How to select tests

- Depends on the diagnostic question
- What evidence is needed to minimize uncertainty and allow action
  - Anatomic, histologic, or clinical pathology correlated with certain disease processes or pathophysiology
  - Exposure or antibody response to pathogen
  - Presence of pathogen, chemical, agent

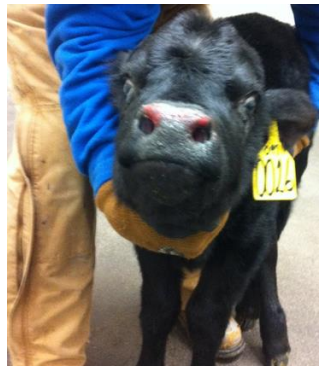






Testing Methods – Diagnostic Question

- What information is needed to decide the next step?
  - Histologic lesions
  - Body system function (dysfunction)
  - Pathogen detection
  - Pathogen isolation
  - Antibody response

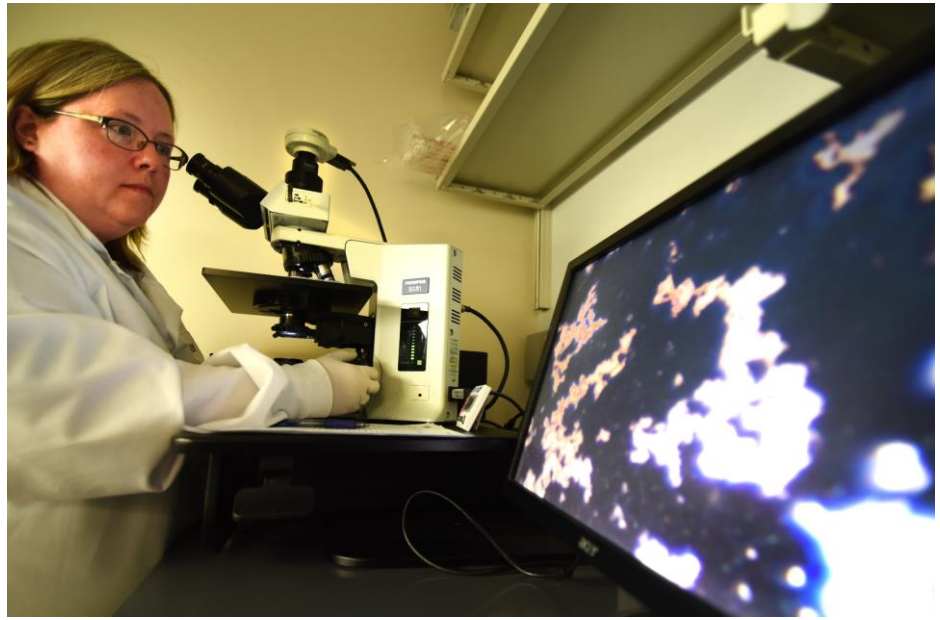


Testing Methods – Diagnostic Question

- What information is needed to decide the next step?
  - **Histologic lesions**
    - Histopathology

Testing Methods – Diagnostic Question

- What information is needed to decide the next step?
  - **Body system function** (dysfunction)
    - Clinical pathology testing
    - Cytology
    - Targeted immunopathology PCRs



Testing Methods – Pathogen Detection

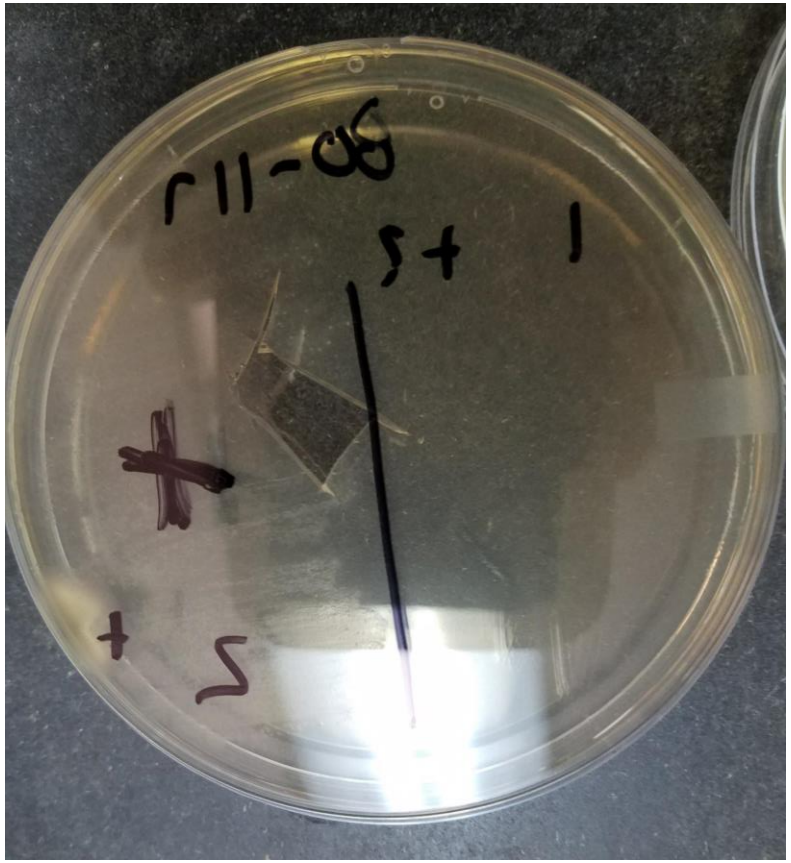
- **<u>Molecular Diagnostics</u>**
  - PCR
  - rt-PCR
- Very sensitive and specific
- Quick answer
- Recent MLV can be detected

Testing Methods – Pathogen Isolation

- **Virus Isolation**
  - Takes longer
  - Dependent on viable virus in the sample
  - Less sensitive than molecular methods
  - Not all viruses can be isolated - BRSV
  - Isolates can be sequenced
  - BRD viral isolates can be from recent MLV

Testing Methods – Pathogen Isolation

- **<u>Bacterial Culture</u>**
  - +/- Takes longer (depends on culture target)
  - Dependent on viable bacteria in the sample
  - Affected by antibiotic administration
  - Antimicrobial susceptibility testing and/or typing PCR can be done on isolates



What test do I want?

- I am unsure of what syndrome to assign to my patient and need to know which organs have been impacted by the disease process
  - Histopathology
- I want to better define the syndromic problem by investigating any pathologic changes in the samples that can be attributed to the syndrome I am investigating
  - Histopathology

What test do I want?

- I want to evaluate the organ/body system function (malfunction) attributed to the syndrome in my patients
  - Chemistry, CBC, Tissue (liver) mineral/heavy metal panel
- I want to look for antibody response to pathogens associated with the current syndrome
  - ELISA, Agglutination tests, Virus Neutralization

What test do I want?

- I want to know if a pathogen is present in the sample (yes/no)
  - rt-PCR
- I want to know what bacteria are present in the sample and want to know the antimicrobial susceptibility +/- send isolates for vaccine development
  - Culture – check what media is required for transport
- I need to be able to differentiate wild type virus from possible MLV vaccine strains
  - VI (sometimes rt-PCR) followed by sequencing

Sample Submission Tips

- **Histopathology**
  - Tissue sections should be ¼" thick and submitted in 10% neutral buffered formalin
  - To assure adequate fixation NBF to tissue ratio should be a least 10:1
  - FIX GASTROINTESTINAL TISSUE AND BRAIN TISSUE ASAP!
  - Protect other samples from formalin fumes during shipment

Sample Submission Tips

- **Clinical Pathology**
  - Serum needs to be separated from RBC and placed in secondary vial ASAP after clotting
  - Prepare slides from whole blood and send with EDTA tube for CBC
    - Protect slides from formalin fumes
  - Protect whole blood from temperature extremes
  - Urine should be sent with cold pack to decrease cell deterioration and bacterial overgrowth
  - CSF is an extremely useful sample in CNS cases

Sample Submission Tips

- **Molecular Diagnostics** (PCR/rt-PCR)
  - Label your swabs!
  - Do not submit swabs in bacteriology media (gel)
  - Do not submit charcoal swabs, cotton swabs, or wood handle swabs
  - Preferred swab type is Dacron or polyester on a plastic handle
  - Keep samples chilled
  - Autolysis negatively affects PCR sensitivity (nucleic acid degradation)

Sample Submission Tips

- **Bacteriology**
  - Label your swabs!
  - Check sample requirements for specific culture needs
  - Provide a history, including animal age
  - Fresh tissues should be kept chilled
  - Success is dependent on sample integrity (probability of live bacteria)
  - Protect samples from formalin fumes

Sample Submission Tips

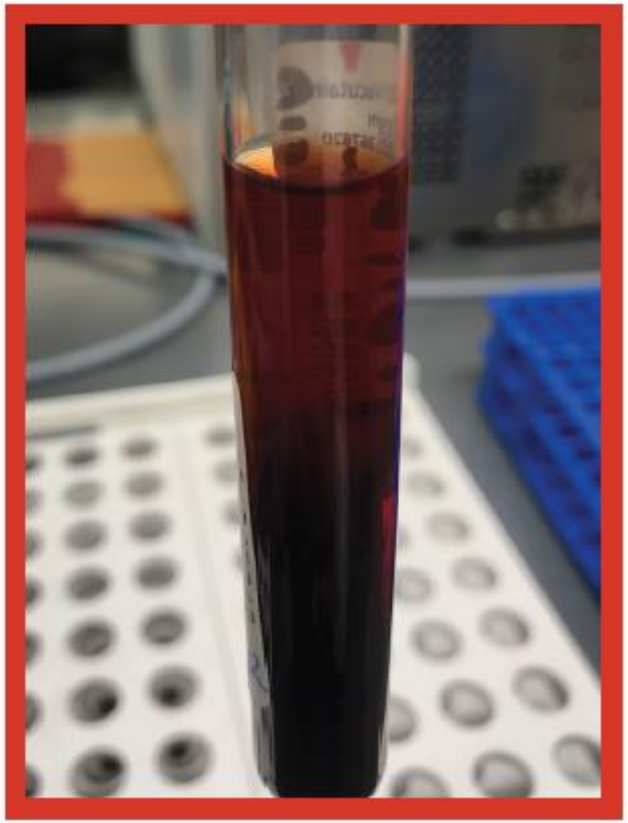
- **Virology**
  - Swabs must be Dacron or polyester and moist on arrival
    - Viral transport media or 0.25 mL PBS/Sterile Saline
  - Fresh tissues should be kept chilled and shipped overnight
  - Autolysis negatively impacts ability to recover virus from sample

Sample Submission Tips

- Drug testing
  - Call the Drug Lab before collecting samples
- Vitamin Testing
  - Protect serum or tissue from light (wrap in foil)
- Tissue ICP/MS Panels
  - Liver for most mineral and metal targets
  - Kidney required for confirmation of lead or copper toxicity
- Serum Mineral Panels
  - Need royal blue top tubes (not red top tubes) for serum
  - Remove serum from RBC before shipment

Serum Sample Submission in General

- Tests may not be run on samples that are lipemic or hemolyzed



Syndromic Testing Plans

TVMDL Bovine Syndromic Plans

- Tests clustered by common syndrome
- Easier test selection
- Entire plan will increase result interpretation potential
- Can add serology panels to most diagnostic plans
- Can customize plans based on pre-test clinical suspicion
  - History, Region, Clinical signs, Exposure risk

General Syndrome Classification Assignments

- Respiratory
- Reproductive
- Digestive
- Weight Loss
- Acute Death
- Neurologic
- Systemic
- Urinary
- Mastitis
- Circulatory
- Endocrine
- Integument
- Musculoskeletal
- Unknown

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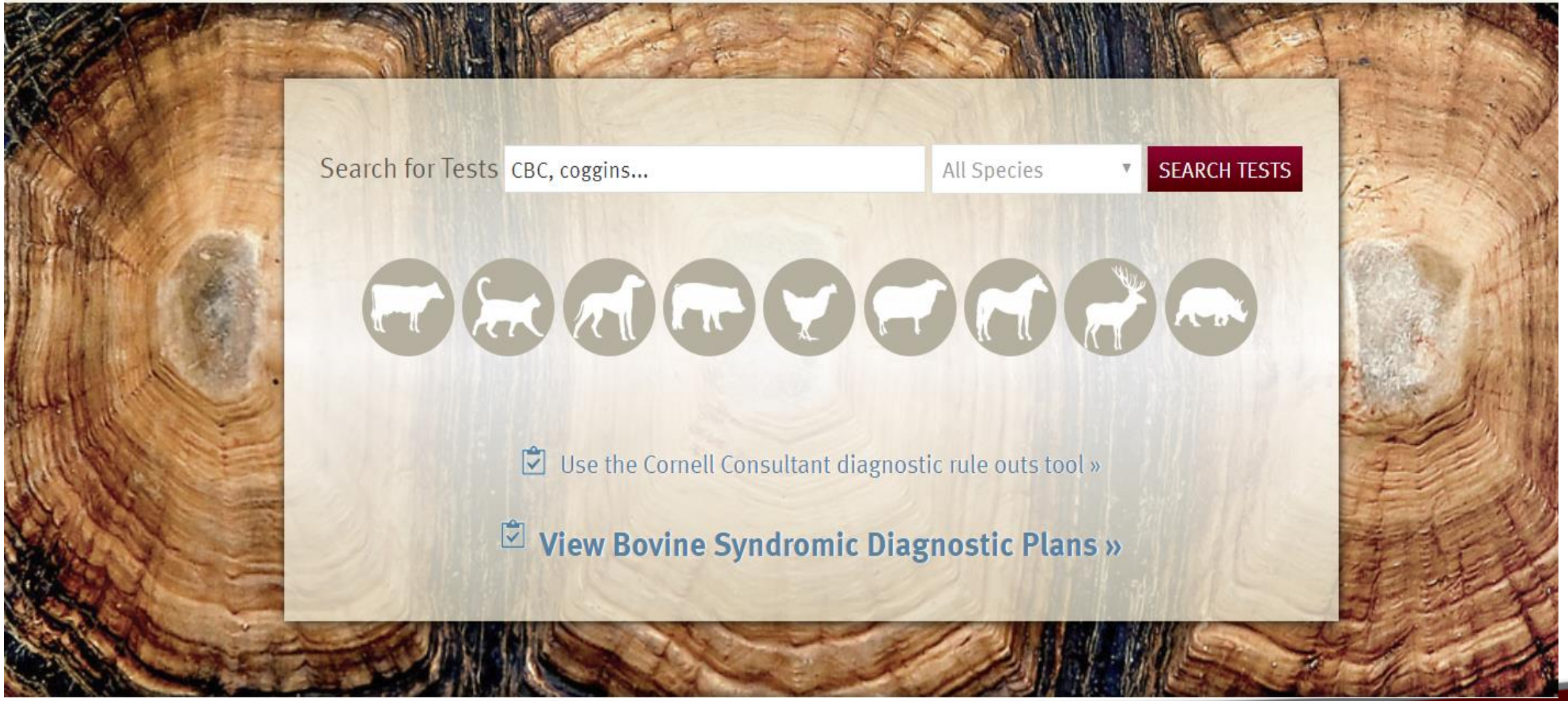
tvmdl.tamu.edu



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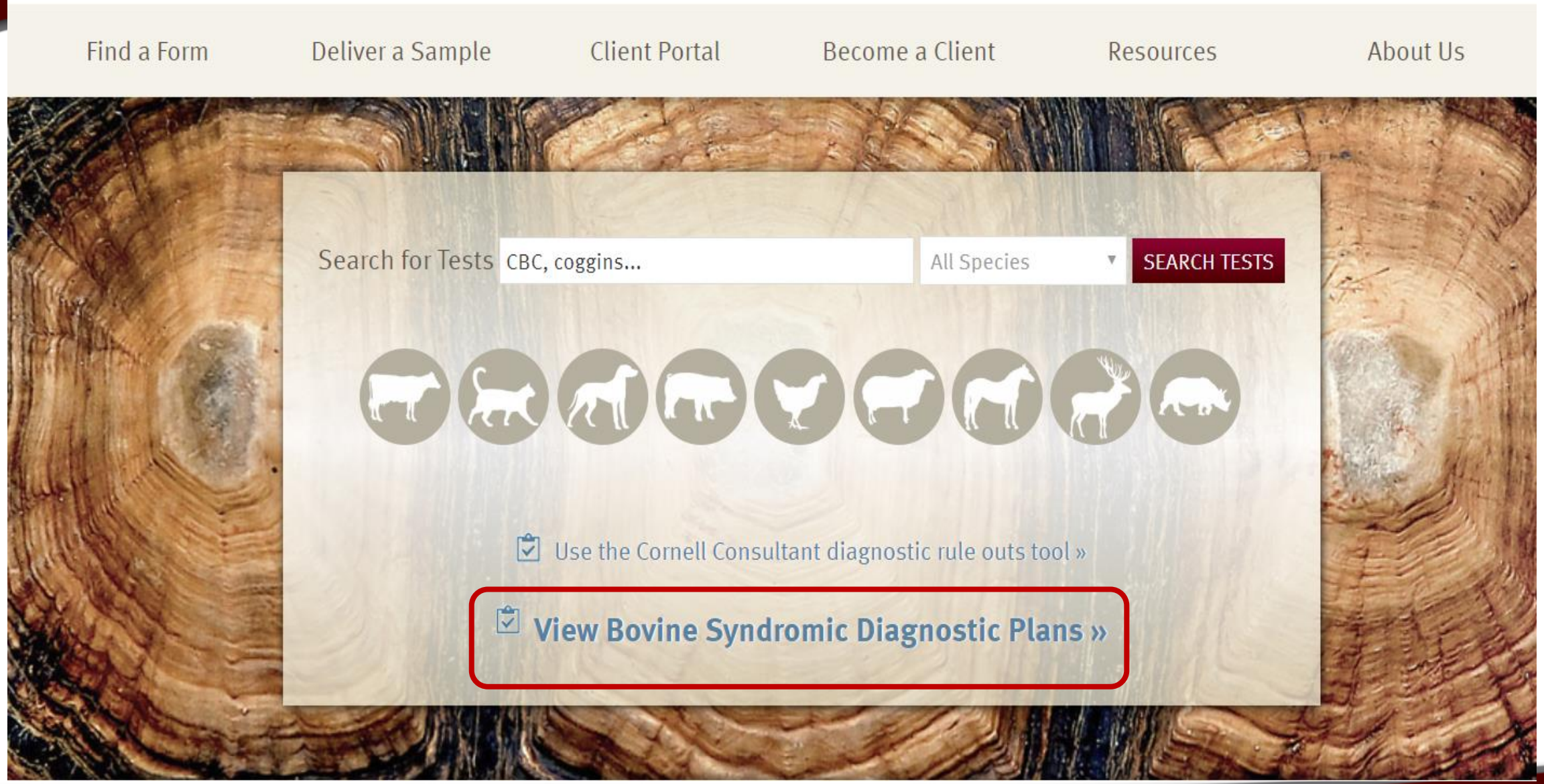

Click below to view each plan:

- Bovine BRD Diagnostic Plan
- Bovine Abortion Diagnostic Plan
- Bovine Calf Diarrhea Diagnostic Plan
- Bovine Adult Diarrhea/Weight Loss Diagnostic Plan
- Bovine Sudden Death Diagnostic Plan
- Bovine Neurological (CNS) Disease Diagnostic Plan
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Bovine BRD Diagnostic Plan

Initial Testing

- Histopathology
- Aerobic & Anaerobic Culture
- Antimicrobial susceptibility
- BRD Bacterial Panel (PCR)
- BRD Viral Panel (PCR)
- Bovine Coronavirus (rtPCR)
- Bovine Influenza D virus (rtPCR)

Secondary/Additional Tests

- Mycoplasma Culture
- BRD Serology Panel (MAT & VN)
- Bovine Coronavirus IFA [Referral]
- Bovine Coronavirus IHC [Referral]

Recommended initial testing:

Test	Samples	Turnaround Time	Section	Lab	Schedule
Histopathology (up to 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AM CS	MTWRF MTWRF
Aerobic & Anaerobic Culture – Livestock	tissues or swabs in Amies media	2-7 days	Bacteriology	AM CS	MTWRFSa MTWRFSa
Susceptibility Test-Food Animal(please indicate MIC or KB preference)	pure isolate	1 day	Bacteriology	AM CS	MTWRF MTWRF
BRD Bacterial Panel - Basic (PCR)	lung, TTW, BAL, nasal/pharyngeal swab	1-3 days	Molecular Diagnostics	AM CS	TWRF TWRF
BRD Viral Panel - Basic (PCR)	lung, trachea, TTW, BAL, nasal/pharyngeal swab	1-4 days	Molecular Diagnostics	AM CS	MTWRF TWRF
Bovine coronavirus (rtPCR)	lung, trachea, TTW, BAL, nasal/pharyngeal swab	1-4 days	Molecular Diagnostics	AM CS	TWRF TWRF
Bovine Influenza D Virus (qPCR)	Lung, trachea, TTW, BAL, nasopharyngeal swab	1-4 days	Molecular Diagnostics	AM	TWRF

Schedule: M=Monday, T=Tuesday, W=Wednesday, R=Thursday, F=Friday, Sa=Saturday

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Histopathology (up to 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AM CS	MTWRF MTWRF
Aerobic & Anaerobic Culture – Livestock	tissues or swabs in Amies media	2-7 days	Bacteriology	AM CS	MTWRFSa MTWRFSa
Susceptibility Test-Food Animal(please indicate MIC or KB preference)	pure isolate	1 day	Bacteriology	AM CS	MTWRF MTWRF
BRD Bacterial Panel - Basic (PCR)	lung, TTW, BAL, nasal/pharyngeal swab	1-3 days	Molecular Diagnostics	AM CS	TWRF TWRF
BRD Viral Panel - Basic (PCR)	lung, trachea, TTW, BAL, nasal/pharyngeal swab	1-4 days	Molecular Diagnostics	AM CS	MTWRF TWRF
Bovine coronavirus (rtPCR)	lung, trachea, TTW, BAL, nasal/pharyngeal swab	1-4 days	Molecular Diagnostics	AM CS	TWRF TWRF
Bovine Influenza D Virus (qPCR)	Lung, trachea, TTW, BAL, nasopharyngeal swab	1-4 days	Molecular Diagnostics	AM	TWRF

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^ View secondary or additional tests

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Test	Samples	Turnaround Time	Section	Lab	Schedule
Histopathology (> 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AMA CS	MTWRF MTWRF
Mycoplasma culture – Livestock	tissues or charcoal swabs in Amies media	14 days	Bacteriology	AMA CS	MTWRFSa MTWRFSa
BRD Serology Panel - Basic (MAT & VN)	serum	3-5 days	Serology & Virology	AMA CS	TF TF
Bovine Coronavirus (IFA Titration)	serum	-	Referral	Infectious Animal Disease Diagnostic Laboratory (Purdue)	-
Bovine Coronavirus IHC	TVMDL tissue blocks	-	Referral	Michigan	-

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BRD Case Submission Pointers

- Do not submit swabs for PCR in gel
- PCR has enhanced sensitivity for viruses and *M. bovis*
- Send second swab if you need culture for AST (susceptibility)
  - 3 swabs if you want Mycoplasma culture
- PCR and VI can detect MLV for up to 4 weeks after vaccination (IBR, BVD)
- BRSV detection LRT samples > URT samples (BAL, TTW)
- Coronavirus detection URT samples > LRT samples (NS, NPS)
  - Be familiar with population benchmarks for shedding vs. clinical syndrome
- Send at least 5 mL serum if asking for numerous BRD antibody tests

BRD Testing Plan Modification – Recent MLV

- rt-PCR and VI can possibly detect virus from MLV
- If rt-PCR panel is Positive for virus of interest and has a low ct value
  - Send for sequencing to compare vaccine vs wild type
- If rt-PCR panel is Positive for virus of interest with a ct value > 30
  - VI sample and if isolate is recovered send for sequencing
- Not 100% validated for BRSV, yet

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Bovine Abortion Diagnostic Plan

Initial Testing

- Histopathology
- Abortion Culture – Livestock
- IBR (BHV-1) rt-PCR
- BVD rt-PCR
- Leptospira spp. rt-PCR
- Neospora caninum rt-PCR
- Liver vitamin A quantification
- Liver trace mineral profile
- (Necropsy)

Secondary/Additional Tests

- B4 Panel (PCR): IBR, BVD, BLV, BTV
- BRD Viral Panel (PCR)
- Anaplasma marginale PCR
- Liver vitamin A + vitamin E
- Nitrates qualitative
- Fungal culture &/or ID
- Comprehensive Abortion Serology
- Listeria monocytogenes PCR [Referral]

Test	Samples	Turnaround Time	Section	Lab	Schedule
Histopathology (up to 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AM CS	MTWRF MTWRF
Abortion Culture - Livestock	fetal tissues, fetal stomach contents, placenta	10 days	Bacteriology	AM CS	MTWRFSa MTWRFSa
IBR (BHV-1) qPCR	lung, trachea, nasopharyngeal swab	1-4 days	Molecular Diagnostics	AM CS	MTWRF TWRF
BVD qPCR	lung, trachea, nasopharyngeal swab, ear notch	1-4 days	Molecular Diagnostics	AM CS	MTWRF TWRF
Leptospira spp. qPCR	kidney, liver, placenta	1-4 days	Molecular Diagnostics	CS	TWRF
Neospora caninum qPCR	brain, placenta, liver, lung, heart	2-3 days	Molecular Diagnostics	CS	TWRF
Liver Vitamin A quantification	10 g liver	1-7 days	Toxicology	CS	R
Liver Tissue Mineral Panel	10 g liver	1-4 days	Toxicology	CS	TR

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View secondary or additional tests

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Histopathology (up to 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AM CS	MTWRF MTWRF
B4 qPCR Panel (IBR, BVD, BLV, BTV)	spleen, liver, lung, lymph node	1-4 days	Molecular Diagnostics	CS	TWRF
Bovine Basic BRD Viral Panel (IBR, BVD, BRSV, PI3)	lung, respiratory swabs	1-4 days	Molecular Diagnostics	AM CS	MTWRF TWRF
Anaplasma marginale PCR	spleen, lung, liver, kidney	1-4 days	Molecular Diagnostics	AM CS	MTWRF TWRF
Liver Vitamin Panel (Vitamin A & E)	10 g liver	1-7 days	Toxicology	CS	R
Liver Single Mineral quantification	10 g liver	1-4 days	Toxicology	CS	TR
Nitrates Qualitative	fetal ocular fluid or eyeball	1-2 days	Toxicology	AM CS	MTWRF MTWRF
Fungal Culture/Identification	tissues, placenta, swabs in transport media	21 days	Bacteriology	AM CS	MTWRFSa MTWRFSa

Bovine Basic Abortion Serology	2 mL serum or fetal effusions	2-3 days 2-4 days	Serology	AM CS	MTWRF MR
Bovine Comprehensive Abortion Serology	2 mL serum or fetal effusions	3-5 days	Serology & Virology	AM	MTWRF
Tirtrichomonas foetus qPCR	1-2 mL fetal abomasal contents or cow cervical wash in trich pouch	2-4 days	Molecular Diagnostics	AM CS	MTWRF MTWRF
Campylobacter spp. qPCR	placenta, lung, 1-2 mL abomasal contents or cervical/uterine wash	1-3 days	Molecular Diagnostics	AM CS	TWRF TWRF
Campylobacter fetus differentiation qPCR	placenta, lung, 1-2 mL abomasal contents or cervical/uterine wash	1-3 days	Molecular Diagnostics	AM CS	TWRF TWRF
Listeria monocytogenes PCR	brain, liver, spleen	-	Referral	KSVDL	-

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Abortion Case Submission Pointers

- Basic Abortion Serology Panel is for herd screening
- Comprehensive Abortion Serology Panel is for investigations
  - Need at least 3 mL serum
- History helps the case coordinators
- Brain is preferred sample type for *Neospora* testing
- Vitamin and mineral abnormalities have been very common this year
- Make sure the organs are in the fetus before submitting to necropsy
- If you submit an entire fetus, there will be a necropsy fee
- Send ear notches for BVD Ag ELISA
- Don’t forget samples for nitrates and Anaplasmosis, just in case

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Bovine Calf Diarrhea Diagnostic Plan

Initial Testing - Feces

- Calf Diarrhea Panel (PCR)
- Aerobic & Anaerobic Culture
- Salmonella genus qPCR
- Salmonella serotyping
- E. coli toxin typing (rtPCR)

Secondary/Additional Tests

- Histopathology
- Antimicrobial susceptibility
- C. perfringens typing (rtPCR)
- Chemistry Profile
- CBC
- Fecal Flotation Qualitative
- McMaster Eggs/Gram
- Electron Microscopy
- BVD Ag ELISA

Test	Samples	Turnaround Time	Section	Lab	Schedule
Basic Calf Diarrhea Panel (Bovine Coronavirus, rotavirus, cryptosporidium)	feces, GI contents, intestines	1-4 days	Molecular Diagnostics	CS	TWRF
Aerobic & Anaerobic Culture – Livestock	feces, fresh tissue	2-7 days	Bacteriology	AMA CS	MTWRFSa MTWRFSa
Salmonella genus qPCR	1 g feces, intestine, fecal swabs	1-4 days	Molecular Diagnostics	CS	TWRF
Salmonella serotyping	salmonella isolate	-	Referral	NVSL	-
E. coli PCR	E. coli isolate	4-6 days	Molecular Diagnostics	AMA	R

Test	Samples	Turnaround Time	Section	Lab	Schedule
Histopathology (> 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AMA CS	MTWRF MTWRF
Susceptibility Test-Food Animal (please indicate MIC or KB preference)	pure isolate	1 day	Bacteriology	AMA CS	MTWRF MTWRF
Clostridium perfringens typing PCR	pure isolate	1-4 days	Molecular Diagnostics	AMA	R
Ruminant Chemistry Profile	0.5 mL serum	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
CBC – Livestock	1 mL EDTA blood + blood film	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
Fecal Flotation Qualitative	3-5 g fresh feces	1-2 days	Parasitology	AMA CS	MTWRF MTWRF
Fecal McMaster EPG (Quantitative)	3-5 g feces	1-2 days	Parasitology	AMA CS	MTWRF MTWRF
Electron Microscopy	feces, GI contents, intestines	5-7 days	Virology	CS	Varies
BVD Antigen Capture ELISA	ear notch, 1 mL serum	1-2 days	Virology	AMA CS	MTWRF TF

Calf Diarrhea Case Submission Pointers

- PCR (Calf Diarrhea PCR Panel) is preferred test for rotavirus, coronavirus, and cryptosporidium
- VI will not isolate (find) coronavirus
- EM will detect rotavirus but it is not as sensitive (or quick) as PCR
- E. coli PCR will type isolates by presence or absence of virulence and toxin genes
  - Will help correlate clinical findings with ETEC, invasive, or other E. coli
- Salmonella PCR + culture with enrichment will increase sensitivity of Salmonella detection in high suspicion cases
- Older calves may need McMaster's EPG and/or Clostridium perfringens testing

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Bovine Adult Diarrhea/Wt Loss Diagnostic Plan

Initial Testing – Feces + Serum

- Aerobic & Anaerobic Culture
- Salmonella serotyping
- Fecal Flotation Qualitative
- Calf Diarrhea Panel (PCR)
- Johne's [*Map*] rtPCR - feces
- BVD Ag ELISA
- Bovine Leukemia Virus (ELISA)

Bovine Adult Diarrhea/Wt Loss Diagnostic Plan

Secondary/Additional Tests

- Histopathology
- Salmonella genus rtPCR
- Chemistry Profile
- Liver Profile
- CBC
- Urinalysis
- Wisconsin Eggs/Gram
- McMaster Eggs/Gram
- Johne's [*Map*] ELISA
- Anaplasma marginale cELISA or PCR
- Tissue Trace Mineral +/- Metal Profile
- Comprehensive Metal Profile – serum/whole blood/plasma
- Bovine Leukemia Virus rtPCR
- Rumen content microscopic analysis

Recommended initial testing:

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Aerobic & Anaerobic Culture – Livestock	feces, fresh tissue	2-7 days	Bacteriology	AMA CS	MTWRFSa MTWRFSa
Salmonella serotyping	salmonella isolate	-	Referral	NVSL	-
Fecal Flotation Qualitative	3-5 g fresh feces	1-2 days	Parasitology	AMA CS	MTWRF MTWRF
Basic Calf Diarrhea Panel (Bovine Coronavirus, rotavirus, cryptosporidium)	feces, GI contents, intestines	1-4 days	Molecular Diagnostics	CS	TWRF
Mycobacterium avium subspecies paratuberculosis (qPCR)	feces, GI contents, intestines	1-4 days	Molecular Diagnostics	CS	TWRF
BVD Antigen Capture ELISA	ear notch, 1 mL serum	1-2 days	Virology	AMA CS	MTWRF TF
Bovine Leukemia ELISA	1 mL serum	1-2 days 1-3 days	Serology	AMA CS	MR TF

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Salmonella culture	feces, tissues	7 days	Bacteriology	AMA CS	MTWRFSa MTWRFSa
Salmonella genus qPCR	1 g feces, intestine, fecal swabs	1-4 days	Molecular Diagnostics	CS	TWRF
Chemistry Profile - Ruminant	0.5 mL serum	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
CBC – Livestock	1 mL EDTA blood + blood film	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
Urinalysis	3 mL urine	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
Wisconsin Eggs/Gram Count (Quantitative)	3-5 g feces	1-3 days	Parasitology	AMA CS	MTWRFSa MTWRF
McMaster Eggs/Gram Count (Quantitative)	3-5 g feces	1-2 days	Parasitology	AMA CS	MTWRF MTWRF
Mycobacterium avium subspecies paratuberculosis (ELISA)	1 mL serum	1-3 days	Serology	AMA CS	TWR MR
Anaplasma marginale cELISA	1 mL serum	1-2 days 1-3 days	Serology	AMA CS	TWR TF
Trace Mineral Panel - Feed/Tissue	10 g liver	1-4 days	Toxicology	CS	TR

Adult Diarrhea/Wt Loss Case Submission Pointers

- Test selection for acute diarrhea will differ than cases of chronic diarrhea and weight loss
- Clinical pathology is very enlightening, especially in cases where individual cows are affected vs. numerous cows in a herd
- Urinalysis is useful for differential list development, especially in older animals
- PCR is preferred test for Johne's disease and Anaplasmosis diagnosis
- BVD Ag ELISA is the most sensitive test for individual animal diagnosis
- Metabolic profiling can be used to assess herd nutrition status

Click below to view each plan:

Bovine BRD Diagnostic Plan

Bovine Sudden Death Diagnostic Plan

Bovine Abortion Diagnostic Plan

Bovine Neurological (CNS) Disease Diagnostic Plan

Bovine Calf Diarrhea Diagnostic Plan

Bovine Pinkeye (IBK) Diagnostic Plan

Bovine Adult Diarrhea/Weight Loss Diagnostic Plan

Bovine Biosecurity Diagnostic Plan

Bovine Sudden Death Diagnostic Plan

Initial Testing

- Histopathology
- Aerobic & Anaerobic Culture
- Anaplasma marginale PCR
- Clostridium FA
- Rumen content microscopic analysis
- Nitrates Qualitative (ocular fluid)

Bovine Sudden Death Diagnostic Plan

Secondary/Additional Tests

- Leptospira spp. rtPCR
- Bovine leukemia virus rtPCR
- Magnesium (ocular fluid)
- Nitrates Quantitative (ocular fluid)
- Cyanide (prussic acid)
- Ammonia (urea/NPN)
- Brain sodium
- Brain cholinesterase
- Tissue Mineral/Heavy Metal Panel
- Toxic Chemical Screen
- Ionophore screen
- Petroleum hydrocarbons
- Blue green algae microscopy
- Chloride
- Rumen pH
- Water Quality [Referral]
- Feed Analysis [Referral]
- Botulism ABC rtPCR [Referral]

Recommended initial testing:

Test	Samples	Turnaround Time	Section	Lab	Schedule
Histopathology (up to 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AMA CS	MTWRF MTWRF
Livestock Culture	tissues, swabs in Amies media	2-7 days	Bacteriology	AMA CS	MTWRFSa MTWRFSa
Anaplasma marginale PCR	spleen, lung, liver, kidney	1-4 days	Molecular Diagnostics	AMA CS	MTWRF TWRF
Clostridium FA	fresh or fixed skeletal mm, cardiac mm, liver	1 day	Bacteriology	AMA CS	MTWRFSa MTWRFSa
Rumen content microscopic analysis	2 cups – 1 quart GI contents	1-5 days	Toxicology	CS	MTWRF
Nitrates Qualitative	1 mL ocular fluid, eyeball, urine, serum, water	1-2 days	Toxicology	AMA CS	MTWRF MTWRF

Schedule: M=Monday, T=Tuesday, W=Wednesday, R=Thursday, F=Friday, Sa=Saturday

Sudden Death Case Submission Pointers

- History is useful to case coordinators
  - Vaccination and other management protocols
  - Feed protocols
  - Herd signalment
- Autolysis limits utility of testing strategies
  - Use the most sensitive tests available
  - Keep possibility of false negatives in mind
- Rumen content, ocular fluid, and feed samples can be collected and sent for secondary testing if needed
- Veterinary diagnosticians and TVMDL toxicologist available for consultation

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- Bovine Biosecurity Diagnostic Plan

Bovine Neurologic Disease Diagnostic Plan

Initial Testing

- Histopathology
- CSF Cytology
- Aerobic & Anaerobic Culture
- Kidney Lead
- Nitrates Qualitative
- Magnesium (ocular fluid)
- Liver vitamins A & E
- McMasters Egg/Gram Coutn
- Listeria monocytogenes PCR [Referral]

Secondary/Additional Tests

- Rabies virus FA [Referral]
- Many other tests that will depend on clinical presentation and animal status

Recommended initial testing:

Test	Samples	Turnaround Time	Section	Lab	Schedule
Histopathology	tissues fixed in NBF	2-5 days	Histopathology	AMA CS	MTWRF MTWRF
CSF Analysis – TNCC	0.5 mL CSF + dried slides	1-2 days	Clinical Pathology	AMA CS	MTWRF MTWRF
CSF Analysis – Microprotein	0.5 mL CSF	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
Aerobic & Anaerobic Culture – Livestock	CSF, brain, CNS tissue	2-7 days	Bacteriology	AMA CS	MTWRFSa MTWRFSa
Lead - Tissue	10 g kidney	1-4 days	Toxicology	CS	TR
Nitrates Qualitative	1 mL ocular fluid, eyeball, urine, serum, water	1-2 days	Toxicology	AMA CS	MTWRF MTWRF
Ocular Fluid Magnesium	ocular fluid or eyeball	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
Liver Vitamin Panel (Vitamin A & E)	10 g liver	1-7 days	Toxicology	CS	R
McMaster Eggs/Gram Count (Quantitative)	3-5 g feces	1-2 days	Parasitology	AMA CS	MTWRF MTWRF
Listeria monocytogenes PCR	brain, liver, spleen	-	Referral	KSVDL	-

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TVMDL Texas A&M Veterinary Medical Diagnostic Laboratory

^View secondary or additional tests

Test	Samples	Turnaround Time	Section	Lab	Schedule
Rabies Testing	brain	-	Referral	TX DSHS	-
Histopathology (> 8 tissues)	tissues fixed in NBF	2-5 days	Histopathology	AMA CS	MTWRF MTWRF
CSF Sodium/Electrolytes, CK &/or Albumin	0.5 mL CSF	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
Liver Mineral/Heavy Metal Panel	10 g liver	1-4 days	Toxicology	CS	TR
Toxic Chemical Screen	rumen contents, liver, forage, hay	1-5 days	Toxicology	CS	MTWRF
Chemistry Profile - Ruminant	0.5 mL serum	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
CBC – Livestock	1 mL EDTA blood + blood film	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
Hemoparasite exam	1 mL EDTA blood, blood film	1 day	Clinical Pathology	AMA CS	MTWRF MTWRF
Ruminant Electrolyte and Macromineral Profile	0.5 mL serum	1-2 days	Clinical Pathology	AMA CS	MTWRF MTWRF
Ruminant Energy Profile	0.5 mL serum	1-2 days	Clinical Pathology	AMA	MTWRF
IBR (BHV-1) qPCR	lung, trachea, nasopharyngeal swab	1-4 days	Molecular Diagnostics	AMA CS	MTWRF TWRF
Anaplasma marginale PCR	spleen, lung, liver, kidney	1-4 days	Molecular	AMA	MTWRF

Bovine Leukemia Virus qPCR	spleen, liver, lymph node	1-4 days	Molecular Diagnostics	CS	TWRF
Botulism ABC rtPCR	rumen contents, feces, spoiled forages	-	Referral	National Botulism Reference Lab	-
Anaplasma marginale cELISA	1 mL serum	1-2 days 1-3 days	Serology	AMA CS	TWR TF
BVD Antigen Capture ELISA	ear notch, 1 mL serum	1-2 days	Virology	AMA CS	MTWRF TF
Fecal Flotation Qualitative	3-5 g fresh feces	1-2 days	Parasitology	AMA CS	MTWRF MTWRF
Lead – Whole Blood	1 mL EDTA blood	1-2 days	Toxicology	CS	MTWRF
Vitamin A-Serum	2 mL serum	1-7 days	Toxicology	CS	R
Brain Cholinesterase quantification	brain	1-3 days	Toxicology	CS	MTWRF
Brain Sodium quantification	fresh brain	2-10 days	Toxicology	CS	MTWRF
Rumen content microscopic analysis	2 cups – 1 quart GI contents	1-5 days	Toxicology	CS	MTWRF
Cyanide (Prussic Acid)	frozen rumen contents	1-2 days	Toxicology	CS	MTWRF
Ammonia (for Urea/NPN toxicity)	frozen rumen contents	1-3 days	Toxicology	CS	MTWRF

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Neurologic Disease Case Submission Pointers

- Indicate rabies suspects clearly on submission form
- Postmortem CSF can be very useful if brain retrieval is not feasible
- Test selection will depend on clinical presentation and lesion localization
- Send in fresh brain if possible – protect from crushing in shipment
- Case consultation before submission is recommended
- Video of animals before death can be helpful for consults

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- Bovine Biosecurity Diagnostic Plan

Bovine Pinkeye (IBK) Diagnostic Plan

Initial Testing

- Aerobic & Anaerobic Culture
- Mycoplasma spp. PCR
- IBR (BHV-1) rtPCR

Secondary/Additional Tests

- Antimicrobial susceptibility testing
- Moraxella speciation PCR [Referral]
- Pinkeye qPCR Panel [Referral]
- Mycoplasma culture

Recommended initial testing:

Test	Samples	Turnaround Time	Section	Lab	Schedule
Aerobic & Anaerobic Culture – Livestock	swabs in Amies media	2-7 days	Bacteriology	AMA CS	MTWRFSa MTWRFSa
Mycoplasma spp. PCR	Dry swab in RTT	3-7 days	Molecular Diagnostics	AMA CS	WR TR
IBR (BHV-1) qPCR	Dry swab in RTT	1-4 days	Molecular Diagnostics	AMA CS	MTWRF TWRF

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View secondary or additional tests

Test	Samples	Turnaround Time	Section	Lab	Schedule
Susceptibility Test-Food Animal (please indicate MIC or KB preference)	pure isolate	1 day	Bacteriology	AMA CS	MTWRF MTWRF
Moraxella speciation PCR	pure isolate	-	Referral	KSVDL	-
IBK qPCR Panel (*Moraxella bovoculi Moraxella bovis, Mycoplasma bovoculi, Mycoplasma bovis* and bovine herpesvirus 1)	eye swab	-	Referral	KSVDL	-
Mycoplasma culture – Livestock	charcoal swab in transport media	14 days	Bacteriology	AMA CS	MTWRFSa MTWRFSa

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Pinkeye Case Submission Pointers

- Please label your swabs
- Sample acute cases before treatment for most relevant information on disease origin
- Sample around lesion rather than the conjunctival recess
- Test selection (PCR vs culture) depends on diagnostic goals
- Indicate on the submission form if you would like susceptibility testing or any isolates forwarded for vaccine development

Questions?

Feel free to contact me with any questions or feedback:

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