Diagnostic Tips for the Equine Practitioner

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1.

COLLECTION CONTAINERS – THE ESSENTIALS

1. Blood collection tubes
   - Red top
   - Purple top EDTA

2. Sterile leak-proof containers
   - Red and white top tubes work for small volume
   - Fecal cup with gasket to prevent spillage for large volume

3. Bacterial transport media
   - Aerobic (Amies)
   - Anaerobic transport media

Bonus: Blood culture bottles, especially if you work with neonates or might work-up a septic joint or meningitis
THE CORNUCOPIA OF BACTERIAL TRANSPORT MEDIA
WHICH TO CHOOSE?

Anaerobic Transport Media

Aerobic Transport Media (Amies)

Blood Culture Bottle

BACTERIAL TRANSPORT MEDIA

Amies aerobic transport media

- Not for anaerobes, even if the label indicates otherwise
  - Human hospitals that plate specimens quickly can use Amies for anaerobes, not vets!

With or without charcoal?

- Without charcoal
  - Works for most aerobic organisms
  - Keeps bacteria alive for 72hrs
- With charcoal
  - Required for CEM cultures
  - Charcoal eliminates metabolic products of bacterial growth – Campylobacter, fastidious growers
BACTERIAL TRANSPORT MEDIA

Anaerobic Transport Media (ATM) – Best kept secret!

Can be used for aerobic, anaerobic, and fungal culture

- 2 different brands
  - ATM
  - BD BBL Port-A-Cul
- 2 different sizes
  - Tube for swab
  - Vial for specimen
- Liquid specimens should be injected directly into the tube or vial through the rubber septum of the cap

THE SECRET SUPERPOWERS OFANAEROBIC TRANSPORT MEDIA

Invisible hydrogen sulfide gas cap inside, heavier than O₂

- Keeps O₂ out to maintain anaerobic environment
- Must hold upright during inoculation so gas cap doesn’t ‘spill out’

Helpful Tips:

- Do not tip tube horizontally during inoculation
- Store at room temperature, don’t refrigerate
- Deliver to lab within 72hrs
COMMON ATM MISTAKES

BLOOD CULTURE MEDIA

Separate bottle for aerobic and anaerobic culture

Also good for culture of fluids of low cellularity

- Joint fluid, CSF

Specimen must be collected using aseptic technique

- Sterile gloves, sterile handling of needle/syringe, sterile prep of collection site required (just like a joint injection)
- Very easy to contaminate blood culture media
BLOOD CULTURE MEDIA

Helpful tips to avoid contamination:
- Clean top of vial with alcohol and let dry prior to inoculation, then cover with sterile gauze while collecting specimen
- Change needle on collection syringe prior to inoculation and between bottles
- Maintain at room temperature prior to submission – do not refrigerate

OTHER TRANSPORT MEDIAS:

BD eSwab™ in liquid Amies media
- Non-enteric aerobic, anaerobic and fungal culture
- Maintains for 48hrs at room temperature
- Labeled for PCR, but not validated for this purpose in most veterinary diagnostic labs

Para-Pak® Fecal Transport Medium
- Contains buffered solution for pH maintenance
- Salmonella
- Shigella
- Yersinia
PLASMA
- Clotting factors present in plasma

Serum
- No clotting factors in serum

Buffy Coat (WBC and Platelets)

The lab can't decipher serum vs. plasma when transferred to a red/white top tube, PLEASE LABEL 😊

RED TOP TUBE

- No additives and sterile
- Plastic red tops are coated with clot activator
- Separating serum avoids hemolysis and artifact on chem panels
  - Serum sitting on cells too long → ↑ K ↓ glucose
  - Certain testing cannot be performed accurately on hemolyzed serum samples
PURPLE TOP EDTA TUBE

- EDTA stands for Ethylenediaminetetraacetic acid.
- EDTA functions by binding calcium in the blood and keeping the blood from clotting.

Common uses:
- CBC
- *Preserve cells for cytology*
  - *i.e. pleural, pericardial, peritoneal, CSF, tracheal wash, bronchoalveolar lavage or joint fluid cytology*
- Endocrinology testing
- PCR on whole blood

- Draw purple tube **after** red top and green heparin tubes used for chemistry analysis to avoid chemistry artifacts.

- EDTA contamination of serum or plasma will cause \( \uparrow \) potassium \( \downarrow \) calcium.
STERILE LEAK-PROOF CONTAINERS

Red and white tops

- No additive
- Uses:
  - Transfer of EDTA/Heparinized plasma
  - Sterile samples (fluid or swab for PCR)

Sterile cup with gasket to prevent spills

MINIMUM DATABASE OF SPECIMENS

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Container</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA Whole Blood</td>
<td>Purple top tube</td>
<td>Refrigerate</td>
</tr>
<tr>
<td>Serum (separated)</td>
<td>Red top tube</td>
<td>Refrigerate</td>
</tr>
<tr>
<td>Manure</td>
<td>Leak proof sterile container</td>
<td>Refrigerate</td>
</tr>
<tr>
<td>Nasal swab or nasopharyngeal wash</td>
<td>Nasal swab: Red top tube with 0.5ml saline Nasopharyngeal wash — sterile leak-proof container like red top tube</td>
<td>Refrigerate</td>
</tr>
<tr>
<td>Nasal swab</td>
<td>Bacterial transport media</td>
<td>Refrigerate</td>
</tr>
</tbody>
</table>
CASE-DEPENDENT - BONUS SPECIMENS

*VERY FRESH MANURE = CAUGHT WHILE PASSED, BEFORE IT HITS THE GROUND IDEALLY

<table>
<thead>
<tr>
<th>Bonus Specimen</th>
<th>Container</th>
<th>Test</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very fresh manure*</td>
<td>Sterile leak-proof container</td>
<td>Clostridium toxin testing</td>
<td>Frozen</td>
</tr>
<tr>
<td>Rectal mucosal swab</td>
<td>Anaerobic transport media (ATM)</td>
<td>Anaerobic culture for Clostridium difficile</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Whole blood</td>
<td>Aerobic and anaerobic blood culture bottles</td>
<td>Blood culture</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Fluids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Tracheal wash</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• BAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Joint fluid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Peritoneal fluid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. EDTA Purple top tube
2. 2-3 unstained air-dried slides
3. Red top tube
4. Anaerobic and anaerobic bacterial transport media

Cytology (1,2)  PCR (3)  Cultures (4)

Refrigerate

CONSIDERATIONS FOR SPECIFIC WORK-UPS

- Abortion
- Respiratory
- Diarrhea
- Neurologic
- Fever of Unknown Origin
- Hepatitis
ABORTION

Common differentials – bacterial/fungal infection, equine viral arteritis, EHV-1, Leptospirosis

Samples
- Dam blood – red and purple top tubes
- Set of tissues
  - Formalin fixed – ok to comingle in jars if contents labeled
  - Package fresh tissues individually
    - Placenta, lung, liver, kidney, stomach contents

Practice Tip: Collect fetal heart blood and fetal effusion
- Used for serology
- Effusion = pleural, pericardial, peritoneal

AHDC EQUINE ABORTION FETAL TISSUE DIAGNOSTIC PLAN

<table>
<thead>
<tr>
<th>Tests Performed</th>
<th>Test Code</th>
<th>Samples Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>(3) Aerobic Bacterial Culture</td>
<td>AER</td>
<td>Submit 3 fresh tissue samples: placenta, lung, and stomach contents - labelled and individually bagged; for individual aerobic culture</td>
</tr>
<tr>
<td>Equine Arteritis Virus (BAV) PA</td>
<td>EVAPA</td>
<td>Fresh tissues: placenta, liver, lung, kidney - labelled and individually bagged</td>
</tr>
<tr>
<td>Equine Herpesvirus PCR Panel</td>
<td>EPVhPA</td>
<td>Fresh tissues: lung (preferred); placenta - use only if lung not available</td>
</tr>
<tr>
<td>Histopathology</td>
<td>HISTO</td>
<td>Formalin-fixed tissues: placenta, liver, lung, brain, adrenal, heart, thymus, small intestine, kidney, and fetal skin</td>
</tr>
<tr>
<td>(2) Leptospira PCR</td>
<td>LEFTPcr</td>
<td>Submit 2 fresh tissue samples: placenta and fetal kidney preferred; stomach contents acceptable</td>
</tr>
</tbody>
</table>

Notes
Collect fetal heart blood, pleural fluid or abdominal fluid and place in red top tube for possible antibody serology testing.
ACUTE NEUROLOGIC WORK-UP

Presentation:
- +/- Fever
- Ataxia

Questions to ask yourself:
*Lack of fever doesn’t rule out infectious etiologies
1. Vaccine status – Rabies, EEE, WN, Tetanus, Botulism?
2. Mosquito exposure? Other seasonal risks?
3. Muscle wasting? If yes, symmetric or asymmetric?
4. Other body systems affected? (Respiratory, GI, Hepatic, Renal)?

FEBRILE ACUTELY NEUROLOGIC HORSE

Thankfully, the preferred diagnostics for most infectious differentials do not require CSF

Serum
- Eastern Equine Encephalitis (EEE) – IgM ELISA
- West Nile Virus (WNV) – IgG/IgM ELISA
- Chemistry
  - Myopathies can sometime be difficult to decipher from other primary neurologic conditions

EDTA whole blood
- EHV1 PCR
- Anaplasma phagocytophilum PCR (pre-oxtetracycline)
- CBC

Nasal Swab in red top with 0.5ml saline
- EHV1 PCR
**AFEBRILE ACUTELY NEUROLOGIC HORSE**

**So many differentials**
- Rabies and many infectious ddx shouldn’t be ruled out without testing

**Also consider:**
- Infectious – EPM, neuroborreliosis, aberrant parasite migration
- Acquired and Degenerative – eNAD, cauda equina, neoplasia, THO, cervical OA
- Congenital – cervical vertebral stenotic myelopathy, cervical vertebral malformation
- Trauma
- Other systemic diseases with neurologic sequelae
  - Renal disease with uremic encephalopathy
  - Liver disease with hepatic encephalopathy
  - GI disease with hyperammonemia (PHF, Coronavirus, Salmonella spp)

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**RABIES**

**Rabie Fluorescent Antibody Test**
- Fresh tissue (not fixed!) in rigid container
- **Cross section of cerebellum and brainstem**
- Inconclusive result if:
  - Sagittal section – lesions can be focal
  - Smooshed specimen
- Send whole head if necessary – let the lab perform brain extraction

ACUTE DIARRHEA WORK-UP

Common differentials

- *Salmonella* spp.
- *Clostridium difficile* and *C. perfringens*
- Potomac Horse Fever (*Neorickettsia risticii*)
- Beta coronavirus
- Cyathostomiasis (small strongyles)

- Additional considerations for foal and weanlings/yearlings
  - Rotavirus (A and B)
  - *E. coli* septicemia
  - *Cryptosporidium* spp.
  - *Strongyloides westeri*
  - *Lawsonia intracellularis*

ACUTE DIARRHEA WORK-UP

- *Salmonella* spp
  - PCR vs Enriched culture with susceptibility?
- *Clostridium difficile* and *C. perfringens*
  - PCR vs anaerobic culture vs toxin ELISA?
- Potomac Horse Fever
  - PCR – whole blood vs feces, or both?
  - Are both *Neorickettsia* spp detected on PCR?
  - When is serum IFA appropriate?
- Rotavirus
  - Not all labs test for both types A and B

[References]

*Identification of a Ruminant Origin Group B Rotavirus Associated with Diarrhea Outbreaks in Foals*

*Real-Time PCR Differential Detection of Neorickettsia findlayensis and N. risticii in Cases of Potomac Horse Fever*
**Species Virulent Toxin genes**

*Clostridium perfringens*  
Type A found in normal and sick foals  
Type C found in sick foals

*Clostridium difficile*  
Toxin A*  
Toxin B*

**Practice Tip**
Equine diarrhea PCR panels are available directly on feces that include *C. perfringens* alpha and NetE/F toxin genes and *C. difficile* toxin A and B genes.

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**Potomac horse fever results (2015-2019)**

**ACUTE RESPIRATORY WORK-UP**

Common differentials diagnosed with PCR of nasal swab or nasopharyngeal wash:

- *Streptococcus equi* subsp. *equi* – ‘strangles’
- Culture and/or PCR?
- Pinnacle MLV detection on PCR - Genotyping
- Equine Influenza A
- EHV-1, EHV-4
- EHV-2 (?)
- Equine adenovirus types 1 and 2
- Equine rhinitis virus types A and B
- Equine Arteritis Virus

Other considerations— bacterial pneumonia, EHV-5 (EMPF), neoplasia, shipping fever

- Tracheal wash or BAL (EMPF)
  - Cytology and culture

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**STRANGLES**

Nasopharyngeal Wash

Practice Tip

- Nasopharyngeal wash is more sensitive than nasal swab for the detection of *Strep equi* subsp. *equi*. If the horse has been recently vaccinated with Pinnacle (within 2-4wks) the PCR may detect vaccine.
- Genotyping from a cultured bacterial isolate is required to differentiate vaccine strain from wildtype *Strep equi* subsp. *equi*
EQUINE HEPATITIS VIRUSES – CORRECTING THE CONFUSION

Since 2011, 4 viruses were described in the context of equine hepatitis:

- Equine pegivirus (Pegivirus E)
- Theiler’s disease associated virus (TDAV, Pegivirus D)
- Equine parvovirus hepatitis (EqPV-H) has been revealed as the cause of Theiler’s disease and mild acute hepatitis
- Equine hepacivirus (EqHV) has been implicated in cases of mild acute and severe chronic hepatitis

Practice Tip:
Only investigate parvovirus and hepacivirus in cases of equine hepatitis

EQUINE HEPATITIS VIRUSES

- PCR on serum is sensitive for screening
  - Equine parvovirus
  - Equine hepacivirus
- Liver biopsy is required to determine if equine hepatitis virus is incidental finding or related to active hepatitis
- Equine parvovirus in-situ hybridization (ISH) on formalin fixed liver biopsies at Cornell AHDC
  - Clarifies the relationship between liver lesions and equine parvovirus

Liver enzyme activity in horses with hepatic necrosis due to equine parvovirus hepatitis

<table>
<thead>
<tr>
<th></th>
<th>Biologic</th>
<th>Non-biologic</th>
<th>Experimental</th>
<th>Reference Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>1187</td>
<td>1070–2423</td>
<td>2505–6239</td>
<td>657</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>114</td>
<td>68–314</td>
<td>115–715</td>
<td>49 (15–233)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>15.1</td>
<td>9.6–24.3</td>
<td>20.1 (8.7–21.2)</td>
<td>19.1 (4–4.3)</td>
</tr>
<tr>
<td>ALP (iu/L)</td>
<td>128</td>
<td>111–171</td>
<td>186 (76–156)</td>
<td>11 (6–148)</td>
</tr>
</tbody>
</table>

Jaeger et al. Wrology Journal (2022) 19:175
### Fever of Unknown Origin

#### Practice Tip – The AHDC equine FUO panel

<table>
<thead>
<tr>
<th>Tests Performed</th>
<th>Test Code</th>
<th>Samples Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplasma phagocytophilum PCR</td>
<td>EHRE</td>
<td>EDTA whole blood in lavender top tube or spleen</td>
</tr>
<tr>
<td>Coronavirus PCR, Beta</td>
<td>BCOR</td>
<td>Fresh feces, colon or colon contents in leak-proof container</td>
</tr>
<tr>
<td>Equine Herpesvirus 1 PCR</td>
<td>EHV1PCR</td>
<td>EDTA whole blood in lavender top tube or spleen</td>
</tr>
<tr>
<td>Equine Herpesvirus 4 PCR</td>
<td>EHV4PCR</td>
<td>EDTA whole blood in lavender top tube or spleen</td>
</tr>
<tr>
<td>Equine Respiratory PCR Panel</td>
<td>ERPNL</td>
<td>Nasal swab or nasopharyngeal swab or oropharyngeal swab or tracheal wash or bronchoalveolar lavage or lung tissue</td>
</tr>
<tr>
<td>Potomac Horse Fever PCR</td>
<td>EHRR</td>
<td>EDTA whole blood in lavender top tube or spleen</td>
</tr>
</tbody>
</table>

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### Influence of Exogenous Steroid Administration on ACTH

- **Joint Injections**
  - Variable duration of effect on baseline ACTH from 2-60 days

- **Long-term systemic steroid administration**
  - Ex. Equine Asthma or IBD cases treated with dexamethasone or prednisolone
  - Stop administration for 2 days minimum or 1 week ideally for baseline ACTH
Influence of Steroid Administration on ACTH

• Use TRH Stim Test to override the negative feedback loop
  • Horse can still respond to TRH with ACTH production

Influence of Stress on ACTH

• ACTH rises during stressful scenarios:
  • Trailering/Travel
  • Veterinary/farrier procedures
  • Sedating a needle-shy horse

• Don’t forget about the ‘internalizer’ – stressed by life, herd dynamic
  • Always interpret your results within the context of the clinical signs and personality of your patient

• The half life of ACTH is 8min
Influence of Stress on ACTH

- Wait **at least** 30min after stressful event before sampling ACTH baseline
- Example:
  - Fjord mare presents to Cornell’s Equine Hospital for history of hoof soreness
  - She was stressed by her trailer ride and **has ongoing stress in new environment**

<table>
<thead>
<tr>
<th></th>
<th>1hr After Hospital Arrival</th>
<th>4hrs After Hospital Arrival</th>
<th>Normal Ref. Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline ACTH</td>
<td>87.6 pg/ml</td>
<td>47.2 pg/ml</td>
<td>9-35 pg/ml</td>
</tr>
</tbody>
</table>

Does this horse really need Prascend?

- Scenario: Horse started on Prascend without baseline testing.
  - Maybe bloodwork was borderline, or horse was stressed during initial testing

- Withhold Prascend for 1 week, then perform TRH response testing to assess PPID status
Withdrawal of Pergolide Prior to Parturition

- **Pergolide** is a dopamine agonist and inhibits prolactin synthesis by lactotrophs in the anterior pituitary → **inhibits lactation**

- Withdraw pergolide 2-4 weeks prior to parturition
  - Personal communication with Boehringer Inghelheim