

Proper Collection and Submission of Samples for Equine Viral Arteritis (EVA) Diagnostic Testing

University of Kentucky-Veterinary Diagnostic Laboratory (UK-VDL)

Introduction:

Equine viral arteritis (EVA) is a contagious viral disease of equids caused by equine arteritis virus (EAV), an RNA virus classified in the family *Arteriviridae*. Only one major serotype of the virus has been identified so far. Equine arteritis virus is found in horse populations in many countries worldwide. Although infrequently reported in the past, confirmed outbreaks of EVA appear to be on the increase.

The majority of naturally acquired infections with EAV are subclinical. Clinical signs of EVA can vary in range and severity. The disease is characterized by fever, depression, anorexia, dependent oedema, especially of the limbs, scrotum and prepuce in the stallion, conjunctivitis, an urticarial-type skin reaction, abortion and, rarely, a fulminating pneumonia, enteritis or pneumo-enteritis in young foals. Apart from mortality in young foals, the case-fatality rate in outbreaks of EVA is very low. Affected horses almost invariably make complete clinical recoveries. A long-term carrier state can occur in a variable percentage of infected stallions.

Purpose:

To obtain the proper specimen for UK-VDL to diagnose EVA by Virus Isolation (VI), PCR or serological methods such as Virus Neutralization (VN) test. Aseptic techniques should be practiced collecting and handling specimens.

Where an outbreak of EVA is suspected, virus isolation (VI) and PCR should be attempted from nasopharyngeal and conjunctiva swabs, blood samples, and semen from stallions considered possible carriers of the virus. Swabs must be placed in viral--*not bacterial*--transport media immediately after collection. Where EVA is suspected in cases of mortality in young foals or older animals, isolation of EAV can be attempted from a variety of tissues, especially the lymphatic glands associated with the alimentary tract and related organs, as well as lung, liver or spleen.

For serological diagnosis of EVA, serum is the specimen of choice. Once collected, the red top should be transported to UK-VDL immediately. If transport is delayed, or sample is mailed, transport with an ice pack to keep specimen cool. A completed EVA form must also accompany the specimen.

Specimen collection:

Swabs: 16" (40 cm) cotton-tipped or dacron-tipped swab or a standard uterine swab can be used. Immediately after collection place swab into a 5-10 ml glass/plastic centrifuge tube (with leak-proof cap) containing sufficient viral transport media to cover the swab tip. *Note—If no transport media is available, sterile saline will work as long as the specimen is kept chilled!*

Blood: For VI, an EDTA (purple top evacuated tube) is preferred. For serological testing, a red top tube is preferred. *Note: blood that is hemolyzed cannot be used for serological testing.*

Note-- *Virus transport medium* is available commercially and generally consists of:

- 1) Phosphate buffered saline (PBS) containing 40% glycerol, 2% antibiotic solution
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- 2) PBS containing 2% tryptose phosphate broth, 2% antibiotic solution (penicillin [10,000 units], streptomycin [10,000 units] in sterile distilled water [100 ml]), and 2% fungizone (250 mg/ml stock).

Procedure for collection:

VN or Virus Isolation

1. Restrain horse.
2. Pass swab at least 6" (15 cm) into the horse's nasopharynx via the ventral meatus to absorb respiratory secretions *for at least one minute*. Insert the swab as far as it will go in the ventral meatus. Avoid the blind pouch just adjacent to the nostril.
3. Cut off cotton-tipped end and drop into the viral transport media.
4. Repeat procedure for other nostril.
5. Label the specimen containers clearly. Fill out an accession form completely, and indicate type of test (e.g. virus isolation or VN). Transport on ice packs (*not wet or dry ice*) to the laboratory as soon as possible. Specify transport media used, and include if it contains antibiotics and/or glycerol.

EVA Serologic testing

1. Blood sample: draw a minimum of 10 ml blood from the jugular vein in an aseptic manner.
2. Complete and EVA regulatory form and send in with the specimen.

Tips for success!

- Make every effort to get the swabs on the first day of clinical signs!
- Be sure to insert the swab at least six inches into the ventral meatus.
- Use enough transport medium to keep the swab thoroughly wet during transport.
- Do not freeze blood samples.