**Enhanced Sensitivity of an Antibody Enzyme-linked Immunosorbent Assay using Equine Arteritis Virus Purified by Anion Exchange Membrane Chromatography**

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### Introduction

An improved competitive blocking ELISA (cELISA) based on a non-neutralizing epitope on *Equine arteritis virus* (EAV) GP5 was previously reported with an initial diagnostic sensitivity and specificity of 99.8% and 95.5% when compared to virus neutralization (VN) test results, respectively. In the field trial performed by AAVLD-accredited state laboratories and OIE reference laboratory, the diagnostic specificity of the same cELISA was 99.5% and the diagnostic sensitivity was 98.2%. The cELISA is not adversely affected by previous exposure of horses to non-EAV biologicals, which cause problems in indirect ELISA and the VN test due to the antibodies against the cell line used in vaccine production.

### Purpose of this study

The current study describes further improvement of the cELISA performance using a novel EAV purification method with anion exchange chromatography (AEC).

### Materials and Methods

- Purification of EAV was performed by a differential centrifugation method and a method using ion exchange membrane capsule. The EAV fractions purified by two methods were analyzed in Western blot and used for preparing cELISA kits.
- Four borderline sensitivity check sets were prepared by diluting EAV-positive sera in negative serum. Another sensitivity check set was prepared with sequentially collected time point sera from horse H681. Horse SR10387 was an EAV carrier, and the remaining four horses (H537, H631, H632, and H681) were vaccinated with EAV MLV (Arvac® b). Four borderline sensitivity check sets determined in VN titer were used to evaluate the analytical sensitivity and specificity, intra-lab repeatability and inter-lab reproducibility when tested with sensitivity check sets (Figure 1).
- AEC-purified antigen cELISA had significantly higher agreement with the VN test than the cELISA derived from differential centrifugation-purified EAV when tested with 43 borderline EAV-seropositive samples as defined by the VN test (Table 2).
- AEC-purified EAV antigen contained ~86.3% GP5 monomer while differential centrifugation-purified EAV contained less than 29.4% GP5 monomer (Figure 2).

### Results

- Improvement of cELISA analytical sensitivity without sacrifice of analytical specificity was clearly evident when cELISAs based on the two purification methods were evaluated using sensitivity check sets (Table 1).
- AEC-purified antigen cELISA had highly significant (p = 0.001) robustness indicated by intra-laboratory repeatability and inter-laboratory reproducibility when evaluated with the sensitivity check sets (Table 1).
- AEC-purified antigen cELISA had significantly higher agreement with the VN test than the cELISA derived from differential centrifugation-purified EAV when tested with 43 borderline EAV-seropositive samples as defined by the VN test (Table 2).
- AEC-purified EAV antigen contained ~86.3% GP5 monomer while differential centrifugation-purified EAV contained less than 29.4% GP5 monomer (Figure 2).

### Conclusion

- The method of EAV purification was successfully developed based on an easily scalable protocol using the AEC.
- Currently, an extensive validation of the improved cELISA to confirm the diagnostic performance, particularly in diagnostic specificity and sensitivity, is ongoing using various sera from horses with MLV vaccination, experimental and natural infections of several EAV strains.
- The improvement of cELISA using anion exchange membrane chromatography may contribute to further harmonize the EAV antibody cELISA with the OIE-prescribed VN test. Furthermore, the results in this and previous manuscripts strongly support the use of this cELISA as a suitable alternative to the OIE-prescribed VN test for serodiagnosis of EAV.

### References