and negative, respectively. Regarding the buffy coat samples, iiRT-PCR detected EIAV nucleic acid in 31/56 AGID positive samples, while 108/109 AGID negative samples tested negative by iiRT-PCR. Total agreement was 84.24%. When whole blood samples were evaluated, a total of 16 iiRT-PCR positive and 15 iiRT-PCR negative out of 31 AGID positive samples were obtained. Additionally, EIAV nucleic acid was not detected in 25 AGID negative samples .Total agreement was 73.21% for this sample type. When evaluating the accuracy of EIAV iiRT-PCR and qPCR, 28/165 and 130/165 buffy coat samples tested positive and negative by both assays, respectively. Five iiRT-PCR positive samples were negative by qPCR and two qPCR positive samples were negative by iiRT-PCR. Thus, EIAV iiRT-PCR showed more than 95% agreement with qPCR results. It has been demonstrated that the absence of clinical signs is correlated with very low, frequently undetectable viremia. Therefore, EIAV iiRT-PCR appears to be a promising tool to identify infected horses including those experiencing low infectivity titers in blood. Furthermore, as initial EIAV replication rates are frequently high, recently infected equids pose a considerable transmission risk long before seroconversion. In our study, a horse was determined seronegative by AGID, but was identified as EIAV infected by iiRT-PCR. Thus, EIAV iiRT-PCR could be considered as an alternative diagnostic tool in the implementation of control strategies during an EIA outbreak in the low prevalence area of our country.

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Equine arteritis virus antibody cELISA, a well-validated alternative to the World Organization for Animal Health (OIE)-prescribed virus neutralization test

C.J. Chung*[1], U. Balasuriya[2], P. Timoney[2], C. Wilson[1], A.L. Grimm[1], K. Pfahl[2], K. Shuck[2], T.C. McGuire[1]
[1] *VMRD Inc., Pullman, Washington 99163;* [2] *University of Kentucky, Lexington, Kentucky 40546*

Equine arteritis virus (EAV) is the cause of equine viral arteritis, a systemic viral disease of equids that is characterized by signs of respiratory disease, abortion, and infrequently, death in young foals. OIE defines a horse as seropositive if its EAV virus neutralization (VN) antibody titer is ≥1:4. The VN test can take up to 72 hours to complete and requires certain laboratory facilities, equipment, and technical expertise to perform. Non-viral cytotoxicity of sera from some horses vaccinated with certain equine herpesvirus-1 vaccines can interfere with interpretation of the test. Inter-laboratory variation in VN results due to variables such as reference virus used, type and cell passage history has been reported. For these reasons an alternative serologic test is desirable, but none of the previously reported ELISA tests (Table 1) have shown equivalent sensitivity and specificity compared to the VN test. With the aim of producing a simpler and faster alternative to the OIE-prescribed test, a cELISA was developed using EAV gp5-specific non-neutralizing monoclonal antibody (MAb) 17B7. This cELISA was validated with diverse sera (n=2469) against the VN test. It had a diagnostic sensitivity of 95.5% and a diagnostic specificity of 99.8% (JVDI. 25:182-8). The MAb-17B7 cELISA was further validated in three EAV-testing laboratories including one OIE-reference and two AAVLD-accredited laboratories: These confirmed a test sensitivity of 99.5% and a specificity of 98.2% (JVDI 25:727-35). As part of test validation, the following five additional analyses were satisfactorily performed according to the OIE-recommended validation protocol: 1. the primary assay was calibrated with the OIE approved reference serum panel for EVA, 2. repeatability of the assay was evaluated within and between runs, 3. analytical specificity was evaluated using sera specific for selected equine viruses, 4. analytical sensitivity was evaluated with sera collected from horses vaccinated with the modified live virus vaccine against EVA (Arvac®, Zoetis Animal Health), and 5. Duration of the positive cELISA antibody was evaluated following EVA vaccination. The analytical sensitivity of the new cELISA was comparable to the VN test in that it detected EAV-specific antibody as early as 6 days post-vaccination. The duration of EAV-specific antibody detection by cELISA was over six years post-vaccination. Based on the data obtained, significant correlation was demonstrated between the VN test and cELISA results (r^2=0.79, P<0.0001). The cELISA was further improved using EAV purified by anion-exchange membrane chromatography (JVDI accepted). This enhanced cELISA was validated using diverse sera (n=3255) at the Maxwell H. Gluck Equine Research Center. The relative sensitivity and specificity of this assay against a group of field sera (n=1851) was 99.6% (95% CI 99.4-100.0) and 98.7% (95% CI 98.3, 99.6), respectively, compared to the VN test (manuscript submitted). This rapid, highly sensitive and specific cELISA compares very favorably to the VN test. It is USDA licensed and should facilitate screening of horses intended for international movement.

Table 1
Diagnostic performance of previous EAV ELISAs

Assay format, number sera tested	Diagnostic sensitivity	Diagnostic specificity	Reference
Indirect ELISA (gp5+N+M), 187	92.3, 57.1%	100%	J Virol Methods 76:127-37
Indirect ELISA (peptide, N and replicase), 200	99%	71%	J Virol Methods 73:175-83
Indirect ELISA (WV), 839	87.5%	98.9%	J Vet Med Sci 60:1043-5
Indirect ELISA (gp5), 1500	99.6%	90.1%	J Virol Methods 54:1-13
Indirect ELISA (WV), 46	96%	26%	Equin Vet J 40(2):182-3
Competitive blocking ELISA (M), 100	86%	100%	CIMID. 26:251-60
Indirect ELISA (gp5), 800	96.75%	95.8%	J Virol Methods 90:167-83
Competitive blocking ELISA (gp5), 675	90.7%	99%	CJVR;64:38-43

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A high-performance multiplex immunoassay for serodiagnosis of flavivirus-associated neurological diseases in horses

C. Beck*[1], P. Desprès[2,3], S. Paulous[3], J. Vanhomwegen[3], S. Lowenski[1], N. Nowotny[4,5], B. Durand[1], A. Garnier[1], S. Blaise-Boisseau[1], E. Guitton[6], T. Yamanaka[7], S. Zientara[1], S. Lecollinet[1]
[1] *UPE, UMR 1161 Virologie, ANSES, INRA, ENVA, ANSES Animal Health Laboratory, EU-RL on equine diseases, 94701 Maisons-Alfort, France;* [2] *UMR PIMIT (I2T team) Université de La Réunion,*

INSERM U1187, CNRS 9192, IRD 249, Technology Plateform CYROI, 97491 Saint-Clotilde, La Réunion, France; [3] *Department of Infections and Epidemiology, Institut Pasteur, 75724 Paris, France;* [4] *University of Veterinary Medicine Vienna, Institute of Virology, Viral Zoonoses, Emerging and Vector-Borne Infections Group, 1210 Vienna, Austria;* [5] *Sultan Qaboos University, College of Medicine and Health Sciences, Department of Microbiology and Immunology, Muscat, Oman;* [6] *INRA UE 1277, Plate-forme d'Infectiologie Expérimentale, 37380 Nouzilly, France;* [7] *Equine Research Institute, Japan racing association, Tochigi 329-0412, Japan*

West Nile virus (WNV), Japanese encephalitis virus (JEV) and tick-borne encephalitis virus (TBEV) are flaviviruses responsible for severe neuro-invasive infections in horses and humans. The confirmation of flavivirus infections is mostly based on rapid serological tests such as enzyme-linked immunosorbent assays (ELISA). These tests suffer from poor specificity, mainly due to antigenic cross-reactivity among flavivirus members. Robust diagnosis therefore needs to be validated through virus neutralisation tests (VNT) which are time-consuming and require BSL3 facilities. The flavivirus envelope (E) glycoprotein ectodomain is composed of three domains (D), e.g. DI, DII and DIII, with EDIII containing virus-specific epitopes. In order to improve the serological differentiation of flavivirus infections, the recombinant soluble ectodomain of WNV E (WNV.sE) and EDIIIs (rEDIIIs) of WNV, JEV and TBEV were synthesised using the *Drosophila* S2 expression system. Purified antigens were covalently bonded to fluorescent beads (figure 1). The microspheres coupled with WNV.sE or rEDIIIs were assayed with about 300 equine immune sera from natural and experimental flavivirus infections and 172 non-immune equine sera as negative controls. rEDIII-coupled microspheres captured specific antibodies against WNV, TBEV or JEV in positive horse sera in a flavivirus multiplex immunoassay (MIA). MIA developed for the detection of flavirus infection showed a relative sensitivity and specificity close to 99% when compared to flavivirus competition ELISA. By comparison with VNT, flavivirus MIA correctly identified the causative flavivirus in 100%, 96.8% and 90.0% WNV-, TBEV- and JEV-infected horses respectively. Several advantages plead in favour of the development and implementation of this flavivirus MIA in reference laboratories: no need for BSL3 facilities to confirm flavivirus infections , requirement of small sampling volumes (1 µl) as well as analysis speed-up (< 3hours).

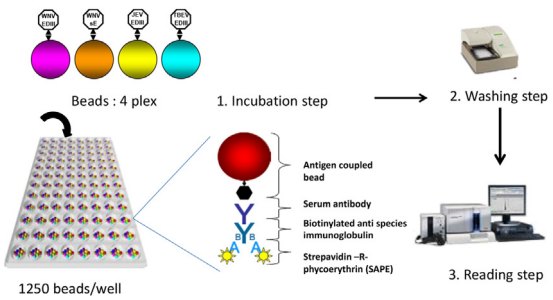

Figure 1. Presentation of the flavivirus MIA with four beads coupled to four antigens (WNV.sE, WNV.EDIII, JEV.EDIII and TBEV.EDIII)

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Development of a Luminex Immunoassay for the serological diagnosis of Vesicular Stomatitis (VS) and Comparison with the Virus Neutralization Test (VNT)

A. Relmy [1], A. Romey [1], D. Toms [2], K. Gorna [1], S. Zientara [1], S. Swenson [2], S. Blaise-Boisseau*[1], L. Bakkali-Kassimi [1]

[1] *Université Paris-Est, Anses, Laboratoire de Santé Animale, UMR1161 Virologie (Anses, Inra, Enva), 14 Rue Pierre et Marie Curie, 94700 Maisons-Alfort, France;* [2] *USDA/APHIS National Veterinary Services Laboratories, Ames, IA, USA*

VS is a zoonotic viral disease affecting livestock. The causative agent, Vesicular Stomatitis virus (VSV), belongs to the genus *Vesiculovirus* in *Rhabdoviridae* family. Two serotypes have been described: New Jersey (NJ) and Indiana (IND), the latter being subdivided into 3 subtypes (Indiana 1, 2, 3). In cattle and pigs, VS remains clinically difficult to differentiate from foot and mouth disease (FMD) when horses are not involved. A prompt laboratory differential diagnosis is thus essential. Serological diagnosis is based on VNT, Complement Fixation and ELISA. This study describes the development of a rapid serological diagnostic test based on Luminex technology and its comparison with the VNT and Competitive ELISA (C ELISA). The objective is to develop a multiplex Luminex test allowing both serotyping and differential diagnosis of VSV. The Luminex technology is based on the principle of flow cytometry and relies on the use of fluorescent microspheres ("beads") which can be coupled to biological macromolecules. A batch of beads was coupled to the envelope glycoprotein of the VSV, serotype NJ (VSV-G-NJ), produced as recombinant antigen using the baculovirus system and harboring a C terminus tag MAT. The coupled beads were incubated with positive equine sera and the antigen/antibody complexes were revealed using biotinylated anti-species antibody and streptavidin-phycoerythrin. Results were analyzed on the Luminex200 platform. Median fluorescence intensity (MFI) values were measured and normalized. VNT were conducted per the OIE manual for terrestrial animals, using the alternative protocol (virus at 100 TCID50 /25µL). Efficiency of antigen/bead coupling was assessed using a monoclonal antibody specific to the MAT tag. Specificity of the coupling was verified using a panel of well characterized positive bovine VSV-NJ- or VSV-IND sera. The protocol was then optimized for equine sera. A prototype test was thus developed, with a cut off value determined using a panel of 100 naive horse sera, in two independent experiments. This prototype was evaluated on a panel of positive equine sera. VNT was performed on the same panel. Results obtained show a good correlation between Luminex, VNT and C ELISA analyses. A first step in developing a Luminex-based serological test for VS diagnostic was reached, using the VSV-G protein-NJ. Similar work is underway using a recombinant VSV-G-IND protein and a duplex test is under development. Bead sets coupled to these VSV-G proteins may also be combined to bead batches developed in our laboratory for FMD serological diagnosis with the aim to offer a differential diagnostic VS/FMD in cattle. The sensitivity and specificity of these luminex prototypes will also be compared to those obtained with the VNT reference method.

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Development and evaluation of a molecular diagnostic method to rapidly detect *Histoplasma capsulatum var. farciminosum* (causing Epizootic Lymphangitis) in equine clinical samples

C.E. Scantlebury [1], G.L. Pinchbeck [1], P. Loughnane [2], N. Aklilu [3], T. Ashine [3], A.P. Stringer [#,4], L. Gordon [2], M. Marshall [2], R.M. Christley [1], A.J. McCarthy [2]

[1] *Institute of infection and global health, University of Liverpool, UK;* [2] *Institute of Integrative Biology, University of Liverpool, UK;* [3] *SPANA*