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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).

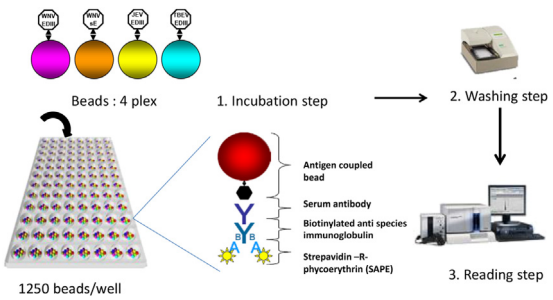


**Figure 1.** Presentation of the flavivirus MIA with four beads coupled to four antigens (WNV.sE, WNV.EDIII, JEV.EDIII and TBEV.EDIII)

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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)

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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

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