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Development of a Bead Based Fully Automated Mutiplex Tool to Simultaneously Diagnose FIV, FeLV and FIP/FCoV

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Abstract : Introduction: Feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), and feline coronavirus (FCoV) are serious infectious diseases affecting cats worldwide. Transmission of these viruses occurs primarily through close contact with infected cats (via saliva, nasal secretions, faeces, etc.). FeLV, FIV, and FCoV infections can occur in combination and are expressed in similar clinical symptoms. Diagnosis can therefore be challenging: Symptoms are variable and often non-specific. Sick cats show very similar clinical symptoms: apathy, anorexia, fever, immunodeficiency syndrome, anemia, etc. Sample volume for small companion animals for diagnostic purposes can be challenging to collect. In addition, multiplex diagnosis of diseases can contribute to an easier, cheaper, and faster workflow in the lab as well as to the better differential diagnosis of diseases. For this reason, we wanted to develop a new diagnostic tool that utilizes less sample volume, reagents, and consumables than multiplesingleplex ELISA assays Methods: The Multiplier from Dynextechonogies (USA) has been used as platform to develop a Multiplex diagnostic tool for the detection of antibodies against FIV and FCoV/FIP and antigens for FeLV. Multiplex diagnostics. The Dynex®Multiplier®is a fully automated chemiluminescence immunoassay analyzer that significantly simplifies laboratory workflow. The Multiplier®ease-of-use reduces pre-analytical steps by combining the power of efficiently multiplexing multiple assays with the simplicity of automated microplate processing. Plastic beads have been coated with antigens for FIV and FCoV/FIP, as well as antibodies for FeLV. Feline blood samples are incubated with the beads. Read out of results is performed via chemiluminescence Results: Bead coating was optimized for each individual antigen or capture antibody and then combined in the multiplex diagnostic tool. HRP: Antibody conjugates for FIV and FCoV antibodies, as well as detection antibodies for FeLV antigen, have been adjusted and mixed. 3 individual prototyple batches of the assay have been produced. We analyzed for each disease 50 well defined positive and negative samples. Results show an excellent diagnostic performance of the simultaneous detection of antibodies or antigens against these feline diseases in a fully automated system. A 100% concordance with singleplex methods like ELISA or IFA can be observed. Intra- and Inter-Assays showed a high precision of the test with CV values below 10% for each individual bead. Accelerated stability testing indicate a shelf life of at least 1 year. Conclusion: The new tool can be used for multiplex diagnostics of the most important feline infectious diseases. Only a very small sample volume is required. Fully automation results in a very convenient and fast method for diagnosing animal diseases.With its large specimen capacity to process over 576 samples per 8-hours shift and provide up to 3,456 results, very high laboratory productivity and reagent savings can be achieved.

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