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Improving African Swine Fever surveillance using fluorescent rapid tests

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Background & Objectives. African Swine Fever (ASF) is a highly infectious disease of swine, caused by the ASF virus (ASFV). Infection with ASFV correlates with a wide range of clinical syndromes from almost unapparent disease to haemorrhagic fever with high fatality rates. To date, there are no commercially available vaccines worldwide and therefore, ASF control is based on early diagnosis and the enforcement of strict sanitary measures. Lateral flow assays are user-friendly, low cost, provide rapid results, and have long-term stability, what makes them one of the most widely used techniques for point-of-care testing, accelerating the final diagnosis.
Materials & Methods. In this work, we described the optimization of two fluorescent lateral flow assays based on the use of europium-labelled nanoparticles. Using specific anti-p72 monoclonal antibodies, a fluorescent lateral flow assay was developed for the direct detection of ASFV antigen (Ag-assay). For the development of the rapid test for the detection of specific ASFV antibodies, p72 ASFV antigen was also bound to europium-labelled nanoparticles (Ab-assay). Analytical sensitivity was determined using a negative blood sample spiked with p72 and with a highly positive serum, respectively. Finally, a group of samples was analysed to determine diagnostic performance. For Ag-assay, a total of 15 experimental positive blood samples were evaluated for sensitivity determination, and 34 field negative blood samples were evaluated for specificity determination. For antibodies detection, a total of 170 experimental positive samples were used to determine sensitivity, and 84 negative field samples (34 blood samples and 50 serum samples) were evaluated to determine specificity. In the following weeks the panel of blood samples for antigen detection will be increased by analysing 100 experimental samples. For the reading of assays' result, an UV-lamp or rapid test reader (Pacific Image Electronics) was used indistinctly.
Results. The optimised assays, exhibited a significant increase in their sensitivity compared to the colorimetric assays, reaching up to a 10-fold increase employing recombinant protein or reference sera, respectively. Finally, assays' performance was determined using the samples described above. For Ag-assay, an increase in assay sensitivity was obtained, especially for samples with Ct values over 30 when compared with the commercial colorimetric assay INgezim® ASFV CROM Ag. Ab-assay also showed an increase in its sensitivity when compared with the commercial assay INgezim® PPA CROM Anticuerpos, and also when compared to the commercial ELISA INgezim® PPA Compac.
Conclusion. The assays described in this work exhibited a huge increase in their sensitivity conserving the advantages of rapid tests. Therefore, they are a useful tool for improving ASFV surveillance through a fast and highly sensitive identification of infected animals, and an active surveillance of serological status.