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Development of a duplex lateral flow assay for simultaneous detection of antibodies against *African* and *Classical swine fever viruses*

Patricia Sastre,[1] Teresa Pérez, Sofia Costa, Xiaoping Yang, Alex Räber, Sandra Blome, Katja V. Goller, Carmina Gallardo, Istar Tapia, Julia García, Antonio Sanz, Paloma Rueda

Abstract. Classical swine fever (CSF) and African swine fever (ASF) are both highly contagious diseases of domestic pigs and wild boar and are clinically indistinguishable. For both diseases, antibody detection is an integral and crucial part of prevention and control measures. The purpose of our study was to develop and initially validate a duplex pen-side test for simultaneous detection and differentiation of specific antibodies against CSF virus (CSFV) and ASF virus (ASFV). The test was based on the major capsid protein VP72 of ASFV and the structural protein E2 of CSFV, both considered the most immunogenic proteins of these viruses. The performance of the pen-side test was evaluated using a panel of porcine samples consisting of experimental, reference, and field sera, with the latter collected from European farms free of both diseases. The new lateral flow assay was able to detect specific antibodies to ASFV or CSFV, showing good levels of sensitivity and specificity. These preliminary data indicate the potential of the newly developed pen-side test for rapid differential detection of antibodies found in the 2 diseases, which is of particular importance in the field and in front-line laboratories where equipment and skilled personnel are limited and control of ASF and CSF is crucial.

Key words: African swine fever; antibodies; classical swine fever; lateral flow assay.

African swine fever (ASF) is an infectious disease of domestic and wild pigs of all breeds and ages, causing a wide range of syndromes from mild disease to lethal hemorrhagic fever.[3,4] The causative agent of the infection, *African swine fever virus* (ASFV; family *Asfarviridae*, genus *Asfivirus*), is a large, enveloped, icosahedral double-stranded DNA virus.[7] The disease is endemic in sub-Saharan Africa and Sardinia.[18,22] ASF has become endemic throughout the Caucasus and the Russian Federation since 2007, where the continued spread poses a serious threat to the swine industry worldwide.[19,25] This was manifested with the introduction of the disease into the European Union member states Poland, Lithuania, Latvia, and Estonia in 2014, where the disease affected both wild boar and domestic pigs.[8] Because no vaccine against ASFV is available, the presence of antibodies against ASFV is used as an indicator of infection.

Classical swine fever (CSF) is a highly contagious disease causing major losses in swine populations almost worldwide.[17] The etiologic agent of CSF is *Classical swine fever virus* (CSFV; family *Flaviviridae*, genus *Pestivirus*), an enveloped, positive-stranded RNA virus.[14] Modified-live vaccines (MLVs) are used routinely in CSF endemic areas, including Asia and central-south America, and induce complete protection against virulent CSFV.[5,13] Although the virus has been eradicated in many countries, the disease has been reported since 2011 in Hungary, Lithuania, Serbia, Israel, the Russian Federation, and Latvia.[23,24]

With the spread of ASFV from the Caucasus, the probability of areas encountering both viruses is increasing, and the presence of wild boar carrying these viruses increases the risk of their introduction into the domestic pig population. In fact, in Latvia, there are overlapping restriction zones for CSF and ASF in wild boar. For these reasons and taking into consideration that both pathogens cause diseases notifiable to the World Organization for Animal Health (OIE), early differential diagnosis is of great value for immediate implementation of control measures to prevent further spread of the diseases. However, differentiation between CSF and ASF by clinical or postmortem examination is currently not possible; therefore, pathogen verification is conducted through laboratory testing.

Inmunología y Genética Aplicada S.A. (INGENASA), Madrid, Spain (Sastre, Pérez; Costa, Tapia, García, Sanz, Rueda); Thermo Fisher Scientific Prionics AG, Schlieren, Switzerland (Yang, Räber); Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany (Blome, Goller); and Centro de Investigación en Sanidad Animal, INIA, Valdeolmos, Madrid, Spain (Gallardo).

[1]Corresponding Author: Patricia Sastre, Inmunología y Genética Aplicada S.A. (INGENASA), C/Hermanos García Noblejas, 41 2º. 28037 Madrid, Spain. psastre@ingenasa.com