

Simultaneous detection of antigen and antibodies to African swine fever virus in a new combo lateral flow assay

Cristina Aira¹, Gabriela González-García¹, Juan Martínez-Cano¹, Nuria de la Roja¹, Ana Ranz¹, Monica Giammaroli², Francesco Feliziani², Žaneta Šteingolde³, Jurate Butkuvienė⁴, Petr Václavěk⁵, Dimitrije Glišić⁶, Carmina Gallardo⁷, Patricia Sastre¹, Marga García-Durán^{1*}, Alba Fresco-Taboada¹ and Paloma Rueda¹

¹ Gold Standard Diagnostics Madrid S.A. (GSD Madrid), 28037, Madrid, España. ² Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" (IZSUM), 06126, Perugia, Italia. ³ Institute of Food Safety, Animal Health and Environment "BIOR", LV-1076, Riga, Letonia. ⁴ National Food and Veterinary Risk Assessment Institute, 08409, Vilna, Lituania. ⁵ Department of Virology, State Veterinary Institute Jihlava, 58601, Jihlava, República Checa. ⁶ Virology Department, Institute of Veterinary Medicine of Serbia, 11000, Belgrado, Serbia. ⁷ European Union Reference Laboratory for African Swine Fever, Centro de Investigación en Sanidad Animal (CISA-INIA/CSC), 28130, Madrid, España.

GARA 2025

Rome, Italy | April 2025

1 INTRODUCTION

African swine fever (ASF) is an infectious disease of swine, which has high fatality rates, and which has a high impact on animal welfare and swine industry. **Since 2018, ASF is actively transmitted around the world.**

Despite all the efforts dedicated to the development of a vaccine, no globally commercial vaccines are available to date. ASF control relies primarily on the **early detection** of infected animals and on the application of strict sanitary measures.

In this context, access to **rapid diagnostic tests** can be of great help in controlling the spread of the disease.

Antigen detection is a direct indicator of infection, while antibody indicates that animals have been exposed to the virus. Therefore, **the combined detection of antigen and antibody is interesting for ASF surveillance**, as it allows the detection of animals in any of the clinical manifestations of the disease and in any point of infection.

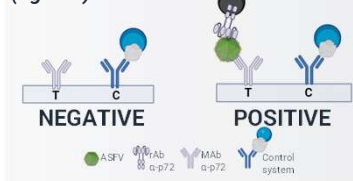
2 OBJECTIVE

Improve **early diagnostic tools for ASF** and develop a combo format for simultaneous antigen and antibody monitoring.

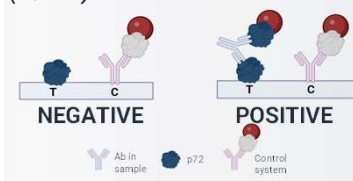


3 MATERIALS & METHODS

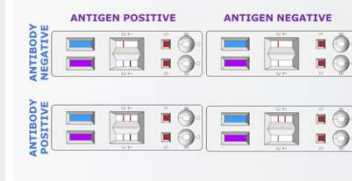
Lateral flow assay for antigen detection (Ag-LFA)



Lateral flow assay for antibody detection (Ab-LFA)



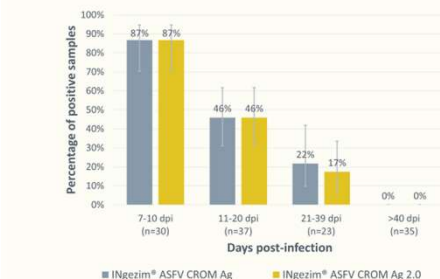
Combo Assay



4 RESULTS

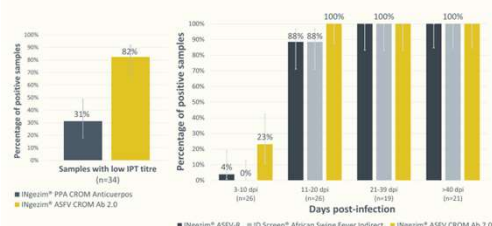
Ag-LFA. INgezim® ASFV CROM Ag 2.0

The antigen detection LFA was improved by using a recombinant antibody specific for p72. The new assay showed the **same sensitivity** (n=125) as the previous commercial test, but with **improved specificity** (98% vs 92%; n=310).



Ab-LFA. INgezim® ASFV CROM Ab 2.0

The antibody detection assay was improved using a new recombinant version of p72. The new assay showed **improved sensitivity** and a 99% specificity (n=298).



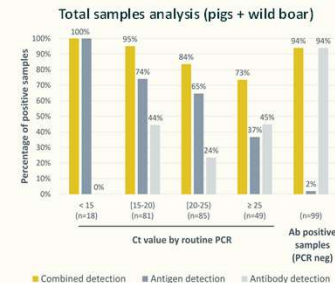
Combined detection. INgezim® ASFV Combo CROM Ag/Ab

A total of 525 blood samples collected from **pigs** and **wild boar** during surveillance campaigns (Latvia, Lithuania, Czech Republic and Serbia) were tested to evaluate the combined test. PCR for antigen detection and ELISA or IPT for antibody detection were used as reference techniques.

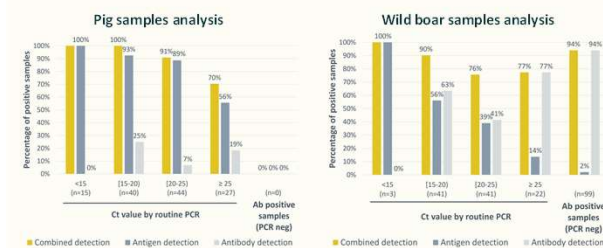
Combined antigen and antibody detection **improved the percentage of positive animals detected.**

Additionally, it allowed the identification of 98 animals that would have been considered negative by antigen detection methods alone.

The assay showed an overall specificity of 97.4% (n=193).



When classifying the samples according to species, a greater advantage was observed for wild boar (n=299).



This observation might be explained by the fact that in this species there is **no control of symptomatology** and, therefore, the animals are found at different stages of the disease.

5 CONCLUSIONS

The **new 2.0 versions** of the rapid assays with recombinant reagents improve the diagnostic parameters.

The **combined detection** of antigen and antibody improves the percentage of positive animals detected in all the groups tested, with a greater improvement observed in samples collected from wild boar.

The results indicate that the new Combo assay can be used with samples collected from both wild boar and domestic pigs, with good results in **both species**.



This work has been partially funded by the **VACDIVA** project. Horizon 2020, Grant agreement ID: 862874.

These results have been published. You can scan the QR code to obtain all the data.

