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Towards the veterinary diagnostics of the future

Main topic : Animal Health

Validation of complete method for the detection of Avian and Swine Influenza Virus (ASIV) genome

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INTRODUCTION

Avian and Swine Influenza (AI and SI) are infectious diseases giving variable symptoms according to the strain pathogenicity and the infected host. They are caused by influenza virus of type A, belonging to the Orthomyxoviridae family. According to the host species, the viruses are considered as avian, swine or other.

Avian influenza is present on the sanitary code list for the terrestrial animals of the Animals World Health Organization (WOAH) and must be declared to the WOAH. Swine and Avian Influenza have a zoonotic potential so monitoring their evolution in animals could help to anticipate human pandemics.

In France, diagnostic tools must be approved by the two National Reference Laboratories for Avian and Swine Influenza virus (NRL, ANSES Ploufragan-Plouzané-Niort, France). Currently, there is no real time PCR detection kit for influenza virus validated by both NRL (AI and SI).

MATERIAL & METHODS

The French AFNOR standard NF U47-600-2 defines the general requirements and recommendations for the development and validation of PCR tools in animal health. These guidelines are included in the two AI and SI NRL specifications, which describe the characteristics and required performances for the release of RT-PCR kits. Upon request, AI and SI NRL supply samples and reference materials at the required detection level.

RESULTS

Bio-T kit® Avian & Swine Influenza Virus V2 is a ready-to-use RT-PCR assay which enables the co-detection of type A Avian & Swine Influenza Virus (ASIV, 6-FAM labelling), an endogenous (IPC, Cy5 labelling) and exogenous (IC, VIC labelling) internal positive control assessing the sample integrity, nucleic acid extraction quality and the absence of PCR inhibitors. The development and validation complied with the following process:
Complete method optimization: RT-PCR and sample processing protocol.
RT-PCR characteristics determination: analytical specificity (inclusivity, exclusivity) and sensitivity (limit of detection).
Assessment of the complete method (from sample to the RT-PCR analysis) for each type of sample and 2 extraction kits (1 magnetic beads system: BioExtract® SuperBall® (BioSellal) and 1 silica column-based system: BioExtract® Column (BioSellal) :

Detection of reference samples corresponding to the minimum required detection level set by the two NRL.
Determination of exact limit of detection using negative samples spiked with avian and swine influenza virus quantified strains.
Evaluation of diagnostic sensitivity and specificity on a panel of known status samples provided by a certified partner laboratory.

DISCUSSION & CONCLUSIONS

The Bio-T kit Avian & Swine Influenza Virus V2 in association with BioExtract® Column and BioExtract® SuperBall® was validated according to the specification of both Avian Influenza and Swine Influenza NRL. The kit showed 100 % of diagnostic sensibility and specificity on an avian influenza field sample panel analysed by a certified partner laboratory, with reproducible results (coefficient of variation below 4% for repeatability and fidelity of the entire method). It provides more accurate results thanks the two internal positive controls: an endogenous guaranteeing the sample quality and an exogenous assessing the absence of RT-PCR inhibitors. Moreover, the exogenous internal positive control allowed the validation of the kit on environmental samples like cloths.