



Development of real-time cell analysis methods applied to equine influenza virus: proof of concept

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Reproducibility was determined in four OIE reference laboratories using the identical assay.

Results: The median diagnostic specificity of the RT-PCR and the ELISA was 99.8% and 98.7% respectively. The median diagnostic sensitivity of the RT-PCR was 99.3% in contrast to 32.2% for the ELISA. There was 100% reproducibility of the assay by the OIE reference laboratories for clinical samples.

Main limitations: Many laboratories internationally were unable to contribute samples.

Conclusions: This validated real time RT-PCR diagnostic assay was approved by the OIE Biological Standard Commission for inclusion in the Terrestrial Manual as suitable for the purpose of certifying horses for trade or movement.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples collected during clinical procedures.

Informed consent: Not applicable.

Competing interests: None declared.

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116 | Development of real-time cell analysis methods applied to equine influenza virus: proof of concept

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Background: Equine influenza virus (EIV) is a respiratory pathogen that causes important economic losses to the equine industry. As a result, equine influenza has benefited from several technological advances in the fields of vaccine development, diagnosis and epidemiological surveillance. New Real-Time Cell Analysis (RTCA) methods (e.g. impedancemetry) allows sensitive measurement of EIV infection, tropisms and replication *in vitro*. This technological approach has now been applied to several equine viruses (e.g. equine herpesvirus; West Nile Virus) but has not been adapted to EIV yet.

Objectives: To develop a RTCA model for EIV.

Study design: *In vitro* experiments, proof of concept.

Methods: 1) Real-time EIV SeroNeutralisation assay (RSNA) was applied to equine serums (n = 5) with different Single Radial Haemolysis (SRH) antibody titres and 2) antiviral compounds activity against EIV were screened. Both assays used MDCK cells. The normalised Cell Index (CIn) was calculated after 30 minutes pre-incubation of EIV A/equine/Jouars/4/2006 (H3N8, Florida Clade 2 sub-lineage) strain

with different equine serums and subsequent cell infection. SRH titres ranged from 0mm² to 252mm². Antiviral compounds Zanamivir and Memantine were used at concentrations ranging from 50 to 1.56 µg/mL. Experimental CIn were compared with control conditions (i.e. cell-culture with/without EIV).

Results: The CIn decrease induced by EIV infection was significantly reduced (p < 0.05) after pre-incubation with the reference EDQM serum (200mm²) and the high SRH titre serums (222 to 253 mm²). Serums with an intermediate or negative SRH titre (123 and 0 mm², respectively) did not prevent the CIn decrease induced by EIV. Zanamivir was significantly active against EIV when used at 12.5 µg/ml (p < 0.05). Memantine was not active against EIV at the concentrations used.

Main limitations: Limited number of serums and EIV strains tested.

Conclusions: RTCA could be used to develop new EIV neutralisation assays and to facilitate the screening of new antiviral molecules.

Ethical animal research: Research ethics committee oversight not currently required by this journal: the study was performed on microbiological samples.

Informed consent: Not applicable.

Competing interests: None declared.

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117 | CRISPR-Cas12a for detection of H3N8 equine influenza virus: preliminary results

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Background: CRISPR-Cas12a, widely used for genome editing, are RNA guided enzymes that bind and cut double-stranded DNA (dsDNA). They possess a collateral cleavage activity on single-stranded DNA (ssDNA), when it is in a context of a ternary complex formed by the Cas12a, a target specific single guided RNA (sgRNA) and the target DNA. This collateral activity can be evidenced by adding a quenched fluorescent ssDNA reporter, allowing development of a sensitive, specific and rapid method for detection of nucleic acids of different pathogens.

Objective: To evaluate CRISPR-Cas12a technology to detect equine influenza virus H3N8 (EIV) in field samples.