40

Simultaneous detection of AIV and NDV by droplet digital PCR (dd-PCR)

Mrs. Alia Yacoub[1]
PhD. Mikhayil Hakhverdyan[1], Prof. Sándor Belák[2],
PhD. Mikael Leijon[1]

[1] *SVA, Uppsala, Sweden*
[2] *SLU, Uppsala, Sweden*

Background
A droplet digital PCR (dd-PCR) evaluated for simultaneous detection of avian influenza virus (AIV) and Newcastle disease virus (NDV). In dd-PCR assay samples are partitioned into 20,000 droplets with target and background DNA randomly distributed among the droplets. After PCR amplification, each droplet provides a fluorescent positive or negative signal indicating the target DNA was present or not present after partitioning. At a certain dilution each droplet provides an independent digital measurement. Positive and negative droplets are counted in a reader and the absolute concentration of target DNA can be calculated.

Methods
The PCR assay presented here is developed using the one-step RT-dd PCR advanced kit for probes from Bio-Rad. Primers and probes for AIV and NDV were from Spackman E. et al 2002 and Wise MG. et al 2004, respectively. RNA from ten AIV strains (H1N1, H2N3, H3N2, H5N1, H5N3, H7N7, H9N2, H13N6, H14N5, H16N3) and from three NDV lentogenic strain LaSota/46 representing genotype II, the velogenic strains Herts´33 representing genotype IV and a pigeon paramyxovirus (PPMV) from Sweden isolated in 2007.

Results
Single dd-PCR and duplex dd-PCR assays were successfully used to detect all AIV strains or NDV strains tested. One-step RT dd-PCR assay was applied for the detection and absolute quantification of NDV and AIV and optimized using serial dilutions of clinical samples from Swedish outbreaks in 2007. Results showed good separation between positive and negative droplets. At the same time, each dilution was quantified. The duplex format was able to differentiate between AIV and NDV with absolute quantification of each virus. The copy numbers of each virus in the duplex dd-PCR were calculated.

Conclusion
A new assay for studying the population/copy number of two different viruses in the same sample was developed. The system could be used for broad detection of different organisms in the same sample and for study the population of each organism at the same time.

249

Syndromic surveillance and pathogen detection using multiplex assays for respiratory infections in small ruminants

Mr. Giovanni Cattoli[1]
Mr. Tirumala BK Settypalli[1], Ms. Kadidia Tounkara[2],
Ms. Angelika Loitsch[3], Mr. Adama Diallo[4],
Mr. Charles Euloge Lamien[1]

[1] *Animal Production and Health Laboratory, Joint FAO/IAEA Division, Seibersdorf, Austria*
[2] *CIRAD, UMR ASTRE, Monpellier, France*
[3] *Institute for Veterinary Disease Control, Austrian Agency for Health and Food Safety (AGES), Moedling, Austria*
[4] *CIRAD, UMR ASTRE, ISRA/LNERV, Dakar-Hann, Senegal*

Background
Several bacterial and viruses can infect the respiratory tract of small ruminants causing similar clinical signs. The differential diagnosis of respiratory diseases in small ruminants can be achieved using multiplex assays for an accurate identification of the causative agents.

Objective
The present study was aimed at developing molecular multiplex assays using different methods, such as real time PCR and microfluidics bead-based technology, applicable for the syndromic surveillance of respiratory infection in small ruminants. The targeted infections were those caused by Capripoxviruses (CaPVs), Peste-des-petits ruminants' virus (PPRV), Parapoxvirus, Mycoplasma Capricolum subsp. Capripneumoniae (MCCP) and Pasteurella multocida (PM). An internal control was included in order to determine the quality of samples being tested.

Methodology
Primers and probes were designed for the conserved regions of the genomes of all the targeted pathogens. The probes for real time PCR were labelled with compatible fluorescent dyes and quenchers, whereas for microfluidics bead based method, primers and probes were biotinylated, phosphorylated and C12 amino-modified accordingly. Total nucleic acid extraction procedures were evaluated to extract both DNA or RNA. The amplification protocols were optimized and the procedures were validated for the amplification of the above-mentioned pathogens in a single test (or tube).

Results
A one-step multiplex real time PCR method was developed to amplify four targets, CaPVs, PPRV, MCCP and PM in order to accommodate real time PCR platforms from different manufacturers and reduce complexity in performing the assay. This real time PCR method was highly specific and sensitive in detecting the targeted pathogens as well as co-coinfections. Out of 314 samples tested from different African countries, 80 samples were positive for PPRV, 50 for PM, 2 for CaPV and 8 were mixed infections of PPRV and PM. The same pathogens were included, and the panel was expanded with Parapoxvirus, an internal control, and tested in microfluidics bead-based method. The validated microfluidics bead-based method displayed a similar analytical sensitivity and specificity to the real time PCR based assay.

Conclusion
The real time PCR method is being implemented in routine diagnostics and surveillance of different veterinary laboratories in Africa and Asia for differential diagnosis of PPR. Microfluidics bead-based assays will extend the scope by allowing the screening of more pathogens. These two multiplex approaches facilitate the syndromic surveillance of respiratory infection in small ruminants in regions where several pathogens with similar clinical symptoms are present.