Rapid detection of Avian Influenza Virus - Towards point of care diagnosis

Bird flu or Avian flu is an infectious disease caused by an influenza A virus of the Orthomyxoviridae family. Avian influenza virus (AIV) causes significant economic losses to the poultry industry worldwide and threatens human life with a pandemic. Pandemic of AIV is the human infection caused by the appearance of a "new" influenza virus as a result of antigenic shift or antigenic drift. Several outbreaks of AIV caused by the rapid spread of infection have been identified. Therefore, there is an urgent need for rapid diagnostic methods that would enable early detection and improve measurements to control the AIV outbreak. Classical method for detection and identification of AIV is time consuming (3-10 days), laborious, less sensitive, and requires special laboratory facilities and trained staff. Molecular diagnostic systems using RT-PCR amplification have significantly improved the speed, sensitivity and specificity of detecting AIV but are still cumbersome, expensive and time-consuming (1-2 days). In both classical and molecular diagnosis, the transportation of sample to the near-by reference or diagnostic laboratory is needed, and this will increases the time for diagnostic result. A simple approach would be to have a point-of-care (POC) diagnostic test at or near the site of sample collection to provide results in very short time and can improve medical decision-making. The available commercial POC tests that are used for screening of influenza A virus are rapid (5-30 minutes) but have low sensitivity and false negative results are of major concern. Ultimately, the miniaturization of the molecular diagnostics using Lab-on-a-chip (LOC) systems could provide the next-generation rapid POC diagnostics. This study has been focused on developing rapid diagnostic methods for the identification and subtyping of the AIV towards POC diagnosis. The first step in molecular diagnostic is sample preparation which is the key to the success of diagnosis. To address this, a novel method was developed for selective separation and purification of AIV from chicken faecal sample using monoclonal antibody (mAb) conjugated magnetic beads where RNA extraction step is not required. The developed bead-based system was able to capture, concentrate and purify all of the 16 H subtypes of AIV from the AIV spiked faecal samples, demonstrating the efficiency of the mAb conjugated beads and the developed method. Subsequently, the newly developed beadiv based method was used in a microfluidic magnetic microsystem for the automation of sample preparation. Using LOC system with a Cyclic-Olefin-Copolymer (COC) polymer chip, the RT-PCR was miniaturized and the entire process was detected in less than 2 h. This integrated LOC system for AIV has a great potential for POC clinical diagnostics. Subtyping of AIV is important in the diagnosis to identify the pathogenic virus. A DNA microarray-based solid-phase PCR approach has been developed for rapid detection of influenza virus types A and simultaneous identification of pathogenic virus subtypes of H5 and H7. This solid-phase RT-PCR method combines a reverse-transcription amplification of RNA extract in the liquid-phase with sequence-specific nested PCR on the solid phase. The examination of 33 avian faecal and tracheal swab specimens was completed in less than 2 h with 94% accuracy. Subsequently, the approach of solid-phase-PCR was extended to a microfluidic chip to reduce sample and reagent consumption. The whole processing time for identifying and simultaneously subtyping AIV was further reduced to 1h. Apart from the RT-PCR method, two immunological methods based on; fluorescent DNA barcode and fluorescent beads were also developed for rapid detection and identification of the AIV. In both methods, the detection involved sandwiching of the target AIV between monoclonal antibodies for nucleoproteins and for matrix proteins. In the fluorescent DNA barcode-based immunoassay, fluorophore-tagged oligonucleotides were used as surrogates for signal detection with sensitivity comparable to conventional RT-PCR for allantoic fluid containing H16N3 AIV. While in the fluorescent bead-based immunoassay, the fluorescent beads were used as the direct detection signal from AIV. In both methods the entire detection time was less than 2 h.

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