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Short communication

Rapid and sensitive detection of infectious bursal disease virus by reverse transcription loop-mediated isothermal amplification combined with a lateral flow dipstick

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A B S T R A C T

Infectious bursal disease (IBD), an immunosuppressive disease that affects all ages of chickens, results in significant losses in the poultry industry. A reverse transcription loop-mediated isothermal amplification (RT-LAMP) combined with a chromatographic lateral flow dipstick (LFD) for the detection of infectious bursal disease virus (IBDV) was developed. The whole process of testing can be completed in less than 70 min using biotin-labeled primers, an FITC-labeled DNA probe, and the LFD. The detection limits for IBDV using RT-LAMP and RT-LAMP-LFD were the same at 10^{-1} plaque forming units (PFU). When other unrelated viruses and cells were tested, no false positive results were observed. In addition, the amplification efficiency of RT-LAMP was enhanced when a loop primer was used. The RT-LAMP-LFD product started to be detected after 40 min. Clinical samples were used to compare assays using RT-PCR, nested RT-PCR, RT-LAMP, and RT-LAMP-LFD and the positive rates were 16%, 40%, 40%, and 40%, respectively. In conclusion, this assay is an easy, rapid, accurate, and sensitive method for the detection of IBDV and will improve the screening of field samples, especially when veterinarians have limited resources.

Infectious bursal disease (IBD) is a contagious and immunosuppressive disease of young chickens. It is caused by infectious bursal disease virus (IBDV), which belongs to the genus *Avibirnavirus* of the Birnaviridae (Azad et al., 1985). There are two serotypes. Serotype 1 is pathogenic and causes severe destructions of the dividing and differentiating B lymphocytes in the bursa of Fabricius. This leads to deficiencies in the humoral immunity and makes birds vulnerable to other bacterial and viral infections (Burkhardt and Muller, 1987). IBD still causes serious economic losses in the poultry industry worldwide (Saif, 1991). IBDV is a non-enveloped icosahedral virus with a diameter of 60 nm and its genome consists of two segments of double stranded RNA (Kibenge et al., 1988). A 110 kDa polyprotein (pVP2–VP3–VP4), which is encoded by an open reading frame (ORF) of segment A is cleaved proteolytically into two structural proteins (VP2 and VP3) and a viral protease (VP4) (Muller and Nitschke, 1987). The 38 kDa viral capsid protein VP2, which can elicit neutralizing antibodies is the primary determinant of viral serotype (Fahey et al., 1989). The 91 kDa VP1 encoded by an ORF of segment B is a double stranded RNA polymerase as well as a capping enzyme and methyltransferase (Spies et al., 1987; Spies and Muller, 1990). To date, immunoassays and molecular biology techniques such as ELISA, agar gel precipitation (AGP), immunofluorescence assays, neutralization assays, probe hybridization, reverse-transcription polymerase chain reaction (RT-PCR), and real-time polymerase chain reaction have been used for the routine diagnosis (Kataria et al., 2001). Most of these methods require a long processing time, the availability of expensive equipments, and trained operators (Liu et al., 2001; Qian and Kibenge, 1996; Stram et al., 1994; Wu et al., 2007).

Recently, a number of auto-cycling cDNA synthesis methods have been invented and applied for the detection of avian pathogens including avian influenza virus (AIV), Newcastle disease virus (NDV), and IBDV (Chen et al., 2008; Curtis et al., 2008; Li et al., 2009; Notomi et al., 2000; Xu et al., 2009; Xue et al., 2009). One of these is RT-LAMP, where the concomitant bindings of four to six primers and the presence of reverse transcriptase together with *Bst* DNA polymerase allow the DNA amplification of a target sequence. After amplification, the restriction enzyme digestion or the nucleotide hybridization can be used to validate the specificity of the RT-LAMP products (Tsai et al., 2009).

In this study, a RT-LAMP combined a lateral flow dipstick (LFD) was developed for the detection of IBDV. RT-LAMP products amplified using biotin-labeled primers were hybridized with a FITC-labeled probe. This hybrid was then bounded dually to the

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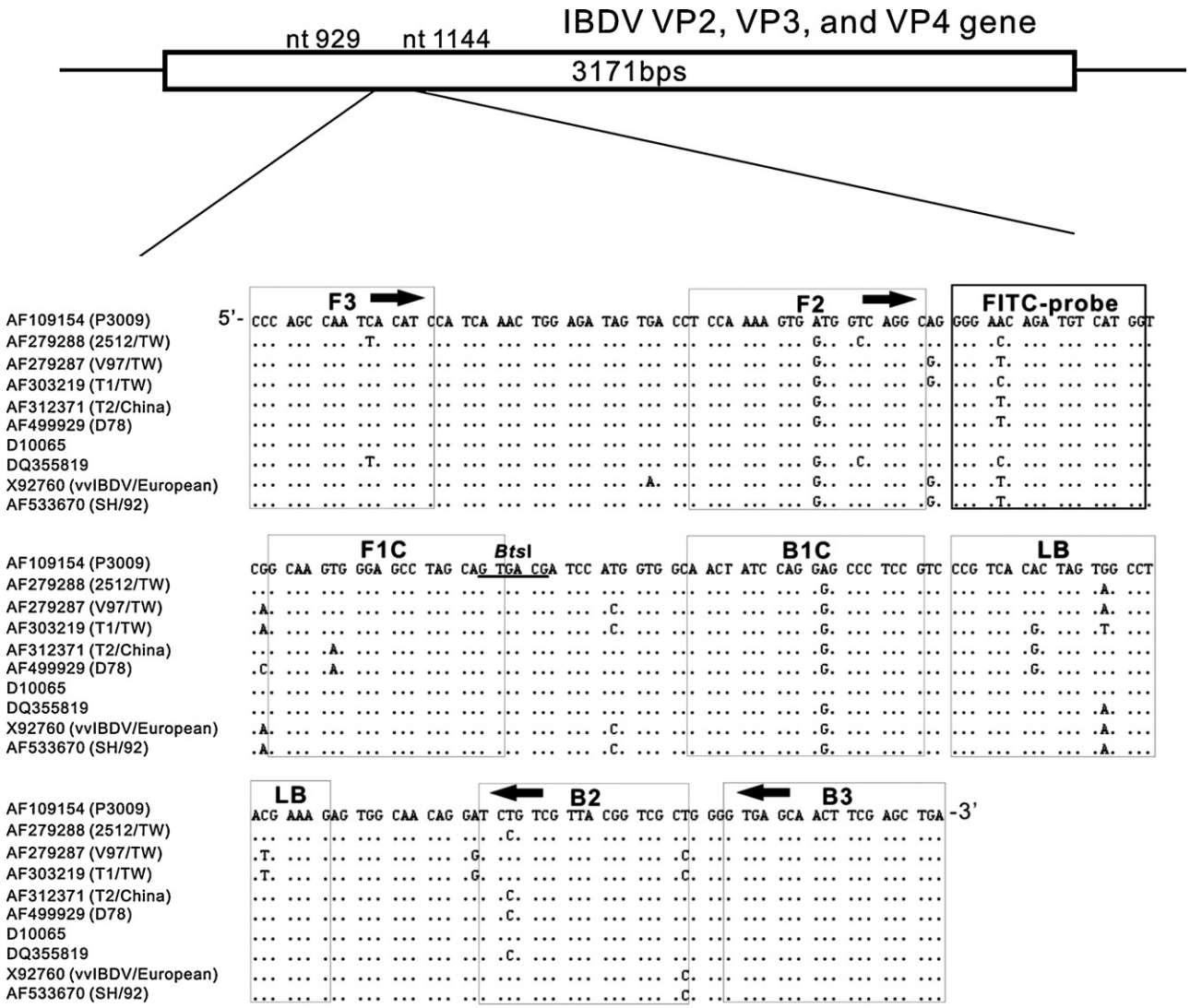

Fig. 1. Sequence alignment of the partial VP2 gene of IBDVs (GenBank accession nos. AF109154, AF279287, AF279288, AF303219, AF312371, AF499929, AF533670, D10065, DQ355819, and X92760). The regions used to design the inner primers (FIP and BIP), the outer primers (F3 and B3), the loop primer (LB), and the FITC-conjugated probe (IBDV-P) as well as the position of a restriction enzyme site (*Bts* I) are denoted. Nucleotide position numbers are primarily based on GenBank accession no. AF109154. Dots are used to represent omitted sequences.

gold-labeled anti-FITC antibody and the anti-biotin antibody coated on a LFD. The final complexes were trapped at the test line of the LFD. Otherwise, without the FITC probe, complexes bound with the gold-labeled anti-FITC antibody were trapped only at the control line. This approach has been used successfully to develop tests for infectious spleen and kidney necrosis virus, shrimp hepatopancreatic parvovirus, shrimp infectious myonecrosis virus, and Penaeus monodon nucleopolyhedronvirus (PemoNPV) (Ding et al., 2010; Nimitphak et al., 2008, 2010; Puthawibool et al., 2009). This system is a user friendly alternative to other techniques and is both rapid and sensitive, while at the same time showing the excellent specificity. This test possesses the great potential when applied in the field.

The P3009 strain of IBDV (GenBank accession number AF109154) was propagated in DF-1 cells cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 5% fetal bovine serum. At 48 hpi, total RNA was extracted using Trizol reagent (MD Bio, Taipei, Taiwan) according to the manufacturer's instructions. For the field samples and other viruses, RNA was also extracted using the above reagent. The cDNA amplification used RNA, 1× RT buffer, 5 units of reverse transcriptase (RTAce, Toyobo, Osaka, Japan), 1 mM each dNTP, 0.4 mM P1 primer, and 0.4 mM P2 primer. PCR or nested PCR was carried out in cDNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.4 mM of both P1 and P2 primers or both P3 and P4 primers, and 0.2 units of Taq polymerase (MD Bio). For synthesis of cDNA, the RT-PCR reaction was performed initially at 42 °C for 1 h and then 94 °C for 5 min followed by 30 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min and a final extension at 72 °C for 10 min. The nested RT-PCR was subjected to 94 °C for 5 min followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min and a final extension at 72 °C for 10 min. Ten microliters of either the RT-PCR or nested RT-PCR products were then separated by 2% agarose gel electrophoresis. The RT-PCR primers (P1 and P2 primers) and the nested RT-PCR primers (P3 and P4 primers) were described by Liu et al. (2001) and Giambrone and Dormitorio (1997), respectively.

The aim of this study was to develop an universal assay for the detection of different strains of IBDV. Selected IBDV strains were used, including several recent Taiwan isolates, vaccine strains, and a number of very virulent IBDV isolates (Liu et al., 2001). Taking into account the fact that the VP2 gene of IBDV is used frequently as the target for the detection of