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Cross-priming amplification combined with immunochromatographic strip for rapid on-site detection of African swine fever virus

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ABSTRACT

African swine fever (ASF) is a highly contagious disease caused by African swine fever virus (ASFV) in domestic pigs and wild boars. Up to now, no commercial vaccines against ASF are available. With the rapid development of international trade and modern logistics and the frequent trade activities with Africa, Europe and neighboring countries, the risk of cross-border transmission of ASF to ASF-free regions is increasing. Therefore, there is an urgent need to establish a convenient and low-cost diagnostic method for rapid and on-site detection of the virus to timely implement the control measures. In this study, a cross-priming amplification in combination with immunochromatographic strip (CPA-strip) was established for rapid detection of ASFV. The CPA-strip assay displayed no cross-reactivity to other swine viruses. The minimum detection limit of this method was 200 copies. Forty-five clinical swine blood samples collected from Uganda were examined by the novel assay, 6 out of 45 samples were tested positive for ASFV. The agreement rate between the CPA-strip assay and the universal probe library-based real-time PCR was 97.8% (44/45). In addition, a total of 100 tissue samples and 57 blood samples from Chinese swine herds were tested to be negative. We concluded that the established CPA-strip method is suitable for on-site detection of ASFV.

1. Introduction

African swine fever (ASF) is a highly contagious disease in domestic pigs and wild boar. The disease is characterized by high fever, cyanosis of the skin, and severe hemorrhages in lymph nodes, with mortality up to 100% [1]. The World Organization for Animal Health (OIE) has included it as a notifiable animal disease, and China has listed it as a predominantly exotic animal disease. ASF is indistinguishable from classical swine fever (CSF) by clinical signs or post-mortem necropsy. African swine fever virus (ASFV), the causative agent of ASF, is a large, enveloped, double-stranded DNA virus, which is the only member of the *Asfarviridae* family. The natural hosts of ASFV are pigs and African soft ticks [2]. Currently, 22 genotypes of ASFV have been identified based on the p72 gene [3].

Since the first report from Kenya in 1921, ASF has spread to dozens of countries [3,4]. In the early 21st century, ASF rapidly spread to Eastern Europe, and later in Russia and other areas, causing significant losses to the pig industry [5]. Moreover, with the frequent trade and modern logistics with countries where ASF is epidemic, the risk of cross-border transmission of ASFV to China is increasing. As there is no treatment or vaccine available for ASF [6], rapid and reliable diagnosis is crucial for the timely implementation of control strategies to prevent the spread of this disease.

The initial OIE-recommended gold standard diagnostic technique for ASFV is virus isolation [7]. However, virus isolation is a time-consuming and labor-intensive assay, which is often used as a confirmatory test in a reference laboratory when ASF is screened by other methods, such as fluorescent antibody test (FAT) [8] and enzyme-linked immunosorbent assay (ELISA) [9]. Recently, molecular diagnostic assays, such as polymerase chain reaction (PCR) [10] and real-time PCR, have been widely used [11]. Considering that antibodies are induced about 14 days post-infection, immunological assays are not suitable for early and rapid monitoring of ASF outbreaks. Though PCR-based technologies are specific and sensitive, yet instruments and techniques are required [12]. Therefore, a rapid, convenient, and on-site method is essential for surveillance of ASF.

Isothermal amplification technologies provide new tools for field diagnosis [13]. Loop-mediated isothermal amplification (LAMP) has been developed for ASFV diagnosis [14]. In addition, cross-priming amplification (CPA) is a novel isothermal amplification technique

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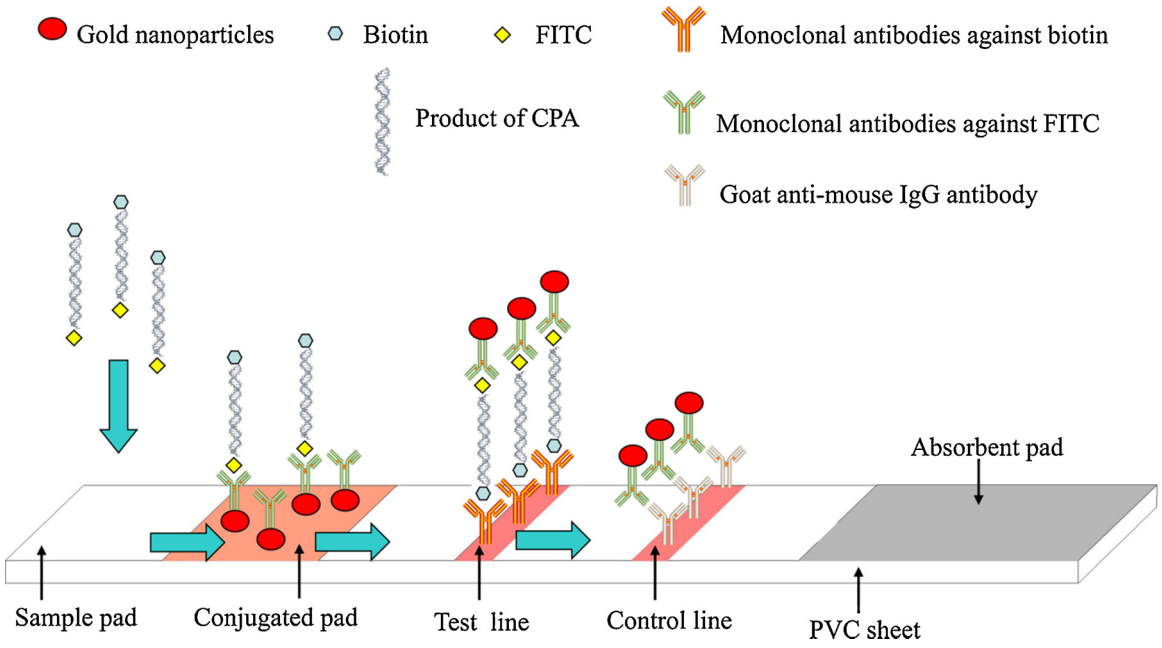

Fig. 1. Schematic diagram of the CPA-strip assay.

developed in recent years [15]. A CPA-based method targeting the ASFV p72 gene has been developed [16]. However, the primers are not highly conserved, making the method less applicable [16]. As a structural protein of ASFV, p54 plays a key role in viral replication and can be used as an indicator to evaluate viral infection [17]. In this study, a novel CPA assay targeting the p54 gene in combination with immunochromatographic strip (CPA-strip) was developed for on-site detection of ASFV. The p54 gene was firstly amplified by CPA using the designed primers and probes and the products were labeled with biotin and FITC at the 5′-ends. Then the amplified products were recognized by anti-biotin and anti-FITC monoclonal antibodies on the test line, where gold nanoparticles (AuNPs) were fixed. Fig. 1 shows the schematic diagram of the CPA-strip assay.

2. Materials and methods

2.1. Virus strains

Several swine viruses were used to evaluate the specificity of the assay, including the classical swine fever virus (CSFV) Shimen strain (genotype 1.1), HLJ strain (genotype 2.1) and the HCLV vaccine strain (genotype 1.1), the highly pathogenic porcine reproductive and respiratory syndrome virus (PRRSV) HuN4 strain, the porcine circovirus type 2 (PCV2) JXL strain, the pseudorabies virus (PRV) TJ strain, SC strain and vaccine strain (Bartha-K61 strain), the porcine parvovirus (PPV) BQ strain, the porcine epidemic diarrhea virus (PEDV), and the bovine viral diarrhea virus (BVDV). The genomic DNA samples of ASFV Lisbon 60, Badajoz 1971 and Port-au-Prince 81 strains were provided by the Swedish National Veterinary Institute (SVA).

2.2. Positive standards

A pair of primers, P54-Sd-F (5′-GAG AAT ACT TGG AAA GTT GGT C-3′) and P54-Sd-R (5′-CGA AGT GCA TGT AAT AAA CG-3′) was designed to amplify the full-length p54 gene fragment by PCR from the genomic DNA of the ASFV E75 strain. The reaction system of PCR was as follows: the 50-μL reaction mixture contained 1 × Ex Taq PCR Mix (TaKaRa, Japan), 0.4 μM primers and 2 μL of the genomic DNA. The PCR amplification products were cloned into the pMD-18 T vector (TaKaRa, Japan), generating pMD-18T-p54, which was used as standards for the assay.

2.3. Design of primers for ASFV CPA

According to NCBI Blast analysis of the ASFV p54 gene sequences, multiple sets of primers and probes were designed in the highly conserved regions based on sequence alignment. The primers and probes were synthesized by Sangon Biotech (Shanghai, China) (Table 1).

2.4. DNA extraction

Viral genomic DNA was extracted from tissue samples using a genomic DNA extraction kit (Omega, USA) according to the

Table 1
The sequences of ASFV CPA primers and probes.

	Names	Sequences (5′-3′)
Scheme 1	P54-5a	CAGACCGGCAACAAACAG
	P54-6s	AGGTCTTTATGCGTATAGGTG
	P54-2a1s	CCAGCAACAAACAAACCAGT-TTT-CCGCCAGTTGCCATGAC
	P54-2a (probe)	**FITC**-CCAGCAACAAACAAACCAGT
	P54-3a (probe)	**Biotin**-GACAACCCAGTTACGGACAG
Scheme 2	P54-5a	GAAGTCACTCCACAACCAGG
	P54-6s	AGGTCTTTATGCGTATAGGTG
	P54-2a1s	CCAGCAACAAACAAACCAGT-TTT-
	P54-4a	CCGCCAGTTGCCATGAC CAGACCGGCAACAAACAG
	P54-2a (probe)	**FITC**-CCAGCAACAAACAAACCAGT
	P54-3a (probe)	**Biotin**-GACAACCCAGTTACGGACAG
Scheme 3	P54-5a	GAAGTCACTCCACAACCAGG
	P54-6s	CCGACATTGTTTGTGAAGC
	P54-2a1s	CCAGCAACAAACAAACCAGT-TTT-
	P54-4a	CCGCCAGTTGCCATGAC CAGACCGGCAACAAACAG
	P54-2a (probe)	**FITC**-CCAGCAACAAACAAACCAGT
	P54-3a (probe)	**Biotin**-GACAACCCAGTTACGGACAG