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Development of a novel protein chip for the detection of bluetongue virus in China

Q.Y. Xu[a], E.C. Sun[a], Y.F. Feng[b], J.P. Li[a], S. Lv[a], Q. Zhang[a], H.X. Wang[a], J.K. Zhang[a], D.L. Wu[a,*]

[a] The Key Laboratory of Veterinary Public Health, Ministry of Agriculture, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, China
[b] Affiliated Hospital of Guangdong Medical University, Zhanjiang 524001, China

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A B S T R A C T

Bluetongue (BT), which is caused by the BT virus (BTV), is an important disease in ruminants that leads to significant economic losses in the husbandry industry. To detect BTV-specific antibodies in serum, a protein chip detection method based on a novel solid supporting material known as polymer-coated initiator-integrated poly (dimethyl siloxane) (iPDMS) was developed. With a threshold of 25% (signal-to-noise percentage), the sensitivity and specificity of the protein chip were 98.6% and 94.8%, respectively. Furthermore, spot serum samples obtained from six provinces of China were tested with the protein chip and a commercially available BTV enzyme-linked immunosorbent assay (ELISA) kit (IDEXX). Of 615 samples, BTV-specific antibodies were detected in 200 (32.52%) by the protein chip and in 176 (28.62%) by the IDEXX BTV ELISA kit. Comparison of the protein chip with the commercial IDEXX BTV ELISA kit yielded the following spot serum detection results: a total coincidence, a negative coincidence and a positive coincidence of 95.12%, 99.28% and 86.5%, respectively. With the protein chip, the BTV-specific serum antibody was detected in samples from all six provinces, and the positive rates ranged from 4.12 to 74.4%. These results indicate that this protein chip detection method based on iPDMS is useful for the serological diagnosis of BTV infection and for epidemiological investigation.

1. Introduction

Bluetongue (BT) is a non-contagious, vector-borne disease in ruminants caused by the BT virus (BTV), which is notifiable in most countries. BTV belongs to the *Orbivirus* genus of the Reoviridae family, and at least 27 serotypes have been reported (Maan et al., 2011; Jenckel et al., 2015). This virus can infect all types of ruminants, and the mortality rate can be as high as 50–70% in certain sheep breeds (Elbers et al., 2008; Mohammadi et al., 2012). BT can cause considerable economic losses due to both the disease itself and the resulting restrictions on international livestock trade. BTV can be transmitted between mammalian hosts by certain biting midges and has been identified in many countries in the tropics, subtropics and temperate zones of Africa, Europe, Asia, North America, South America and Oceania (Maclanchlan, 2011), although it is primarily observed between the latitudes of 40°S and 53°N (Pathak et al., 2008). BTV was first identified in Shizong County of Yunnan Province of China in 1979, and currently, at least seven serotypes of BTV have been isolated (Zhang et al., 2004). The genome of BTV is composed of ten linear segments of double-stranded RNA encoding seven structural and four nonstructural proteins. VP7 comprises approximately 36% of the total virion content and is the major group-specific antigen (Yang et al., 2009; Wang et al., 2012; Pathak et al., 2008). VP7 of BTV and VP7-specific antibodies are usually utilized for detection of the virus because VP7 is highly conserved among variants of BTV. Immunofluorescence, agar gel immunodiffusion (AGID), enzyme-linked immunosorbent assay (ELISA), the immunospot test and indirect peroxidase/antiperoxidase identification are commonly used methods for the identification of BTV infection at the serogroup level (Dadhich, 2004). The use of protein chips is a promising approach for a wide variety of applications, including the identification of protein-protein interactions, protein-phospholipid interactions, small-molecule targets, and substrates of protein kinases. Protein chips can also be used

* Corresponding author.
E-mail addresses: x_qingyuan@163.com (Q.Y. Xu), ecsunhvri@163.com (E.C. Sun), fyf_anna@163.com (Y.F. Feng), lijunping916@163.com (J.P. Li), shuanglv8728@163.com (S. Lv), 472764575@qq.com (Q. Zhang), 403584095@qq.com (H.X. Wang), 15776628009@163.com (J.K. Zhang), dlwu@hvri.ac.cn (D.L. Wu).

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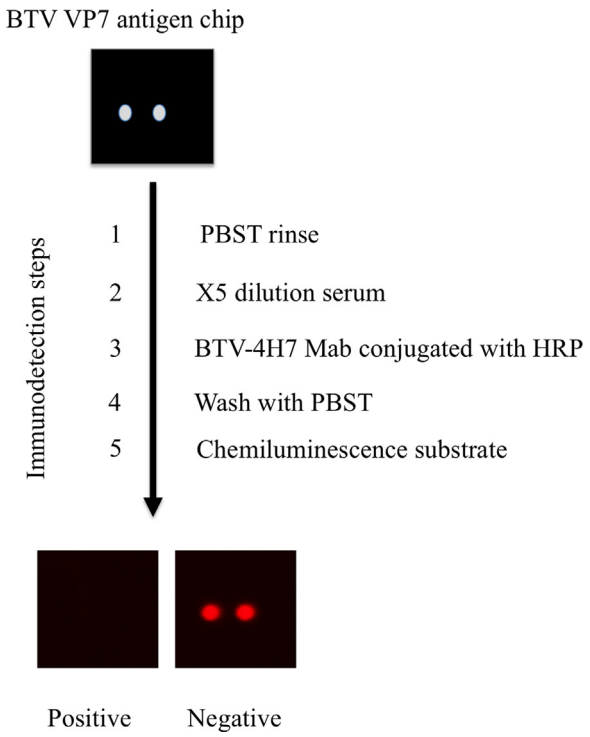

Fig. 1. Operational procedure for the protein chip.
Step 1, the antigen-bound chip was rinsed by moistening with PBST for 5 min; Step 2, 75 μL of 10-fold-diluted serum was added and incubated for 30 min on a temperature-controlled shaker; Step 3, 75 μL of Mab conjugated to HRP was added, and the plate was incubated for 30 min on a temperature-controlled shaker; Step 4, the plate was washed with PBST; Step 5, 15 μL of chemiluminescence substrate was added to each well, and the relative light units were measured with a luminometer.

for clinical diagnostics and for monitoring of disease states (Wang et al., 2010). Protein chips specifically allow for fast, easy and parallel detection of thousands of addressable elements in a single experiment. However, nonspecific protein adsorption, which usually leads to high background, is a major obstacle in developing more sensitive and specific protein chips. To overcome this problem, several substrates have been developed, including poly(dimethylsiloxane) (PDMS), nitrocellulose (NC), polystyrene, poly(methyl methacrylate) (PMMA), nylon, poly(vinylidene fluoride) (PVDF), poly-L-lysine (PLL), polyelectrolyte thin films and polymer-coated initiator-integrated PDMS (iPDMS). iPDMS is a novel substrate created through functional surface modification of PDMS with polymer brushes of oligo(ethylene glycol)methacrylate, and nearly "absolute zero"-level nonspecific protein adsorption can be achieved with this material.

Here, we describe a novel BTV protein chip detection method for VP7-specific serum antibodies based on the "zero background" iPDMS substrate. The method was subsequently applied to evaluate the seroprevalence of BTV in serum samples collected from goats and sheep in several provinces of China.

2. Materials and methods

2.1. Serum, protein, and antibody

A total of 614 serum samples were collected from goats or sheep in Liaoning, Tianjin, Sichuan, Fujian, Inner Mongolia and Xinjiang Provinces of China in 2014.

Standard serum samples from animals infected with BTV-1, BTV-2, BTV-3, BTV-4, BTV-5, BTV-6, BTV-7, BTV-8, BTV-10, BTV-11, BTV-13, BTV-14, BTV-15, BTV-16, BTV-17, BTV-18, or BTV-20 and strongly and weakly positive serum samples were purchased from Beijing Laboratory Biology Technology Co., Ltd., China.

Positive and negative reference sera were prepared by combining 20 positive and 20 negative serum samples, respectively, at equal volumes.

Four challenged sheep serum samples and one control sheep serum sample, which were collected at regular time intervals, were preserved in the laboratory. The purified VP7 protein (600 μg/mL) and the group-specific activity-blocking Mab BTV-4H7 (3170 μg/mL) were conserved in our laboratory (Xu et al., 2015). The purified BTV-4H7 Mab was conjugated to horseradish peroxidase (HRP) using a Lightning-Link® HRP conjugation kit (Innova Biosciences, UK) according to the manufacturer's instructions. Briefly, 10 μL of LL-modifier reagent was added to 100 μL of Mab and gently mixed. The Mab was subsequently transferred into the Lightning-Link mix and gently resuspended by withdrawing and re-dispensing the liquid twice, and the mixture was then incubated at 4 °C overnight. A total of 10 μL of LL-quencher reagent

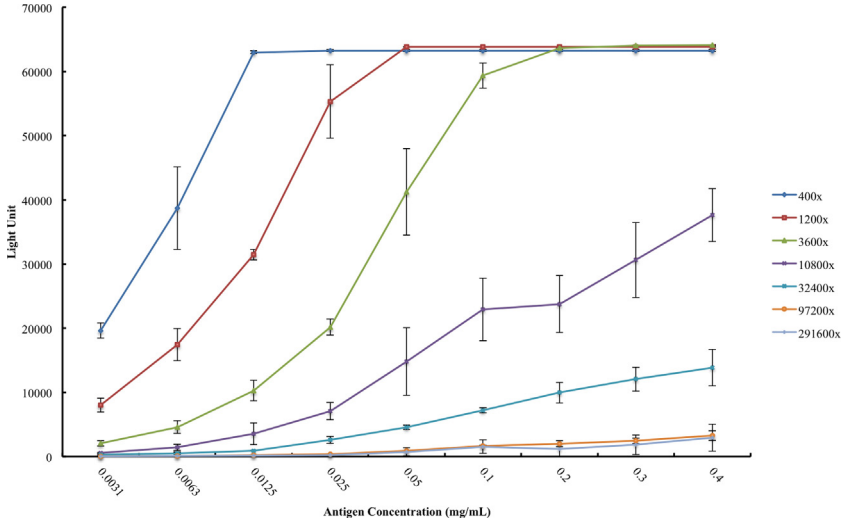

Fig. 2. Optimization of the antigen and HRP-conjugated Mab concentrations based on a chessboard assay.
A chessboard assay was applied to identify the optimal antigen and Mab concentrations for the protein chip. As shown in the figure, a plateau was achieved with 0.2 mg/mL antigen and 3600× Mab. The original concentration of HRP-conjugated Mab was 3170 μg/mL, and 0.88 μg/mL in the protein assay.