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Short communication

Colloidal gold-based immunochromatographic strip test compromising optimised combinations of anti-*S. suis* capsular polysaccharide polyclonal antibodies for detection of *Streptococcus suis*

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ABSTRACT

A rapid diagnosis kit that detects *Streptococcus suis* (*S. suis*) antigens from urine with an immunochromatographic stripe (ICS) test was developed in this study. The ICS test was produced using colloidal gold coated with polyclonal antibodies (pAbs) against *S. suis*. The pAbs were developed from rabbits immunised with *S. suis* serotype 2 capsular polysaccharides (CPS). Development of the pAbs was investigated to establish their binding to CPS and to determine the maximum sensitivity of two combination antibodies for the ICS test. The results of the ICS optimisation revealed that the combinations of pAb C-N1 and pAb C-N2 had the highest sensitivity to CPS. The minimum limitation of ICS sensitivity indicated 1.0×10^4 colony forming units (CFU) and a CPS concentration of 0.05 μg. The assay time for detection of *S. suis* antigens is less than 15 min, which is suitable for rapid detection. A cross-reactive test was also conducted, and it detected no other bacteria (*Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*). The cross-reactivity of other serotypes in *S. suis* was also investigated, and tests for serotypes of 1, 1/2, 3, 4, 5, 6, 7, 8, 9, 14, and 16 were positive. This study presents the first report of a development of an ICS that enables the quantitative detection of streptococcal antigens. The *S. suis* ICS provides several advantages over other methods, including the speed and simplicity of use.

1. Introduction

Streptococcus suis is a Gram-positive and facultative anaerobe bacteria and is associated with a wide range of diseases in pigs and humans, including meningitis, septicaemia, pneumonia, endocarditis, and arthritis. There are 35 serotypes of *S. suis*, and serotype 2 is the most famous virulent strain (De-Greeff et al., 2002). In human infectious epidemiology, the first human case of *S. suis* infection was reported in Denmark in 1968 (Staats et al., 1997). In 2005, a large outbreak of human *S. suis* infection occurred in Sichuan, China, involving more than 200 cases with 38 deaths (Yu et al., 2006). Recently, there have been some reports of persons infected with *S. suis* in Thailand (Kerdsin et al., 2011a), Vietnam (Wertheim et al., 2009), and Hawaii (Fittipaldi et al., 2009).

Diagnosis of *S. suis* infection is basically conducted as a traditional microbiological and biochemical analysis. However, traditional methods are routine and complex, so they are sometimes not accurate enough and fail to properly diagnose infection. Several biological molecular techniques have been developed. An PCR assay designed for the amplification of specific *S. suis* genes has been successfully demonstrated to detect *S. suis* (Kerdsin et al., 2011b). Furthermore, the loop-mediated isothermal amplification (LAMP) assay is one of the most convenient tools for diagnosis. *S. suis* can be detected at the low concentration of 10^2 colony forming units (CFU) (Huy et al., 2012). However, these molecular techniques require expensive equipment and highly skilled personnel. Thus, diagnosis methods based on molecular techniques are not suitable for small hospitals or for field diagnoses.

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Although the immunochromatographic stripe (ICS) is a highly convenient tool for detecting bacteria and bacterial antigens (Pengsuk et al., 2013), there is still no report on immunochromatography developed for the detection of *S. suis* antigens.

Urinary antigens have been widely used for bacteria diagnosis, such as for *Legionella pneumophila* (*L. pneumophila*) (Helbig et al., 2001), *Streptococcus pneumoniae* (*S. pneumoniae*) (Picazo et al., 2013), and *Helicobacter pylori* (Okuda et al., 2013). Detection of urinary antigens of *S. pneumoniae* forms the basis of a major diagnosis ICS kit, Binaxnow, which has been commercialised all over the world. This kit captures streptococcal capsular polysaccharides (CPS) from urine. *S. suis* is one of the species in the *Streptococcus* genius, and it also has capsular polysaccharides. In our previous study on mice, *S. suis* CFU were detected from mouse urine after *S. suis* infection (Nakayama et al., 2011). These findings suggest that it might be possible to detect *S. suis* antigens from urine and thus diagnose *S. suis* infection from urine samples. The aim of this study is to develop an ICS against *S. suis* serotype 2 and to detect *S. suis* antigens in urine.

2. Materials and methods

2.1. Bacterial strains and cultures

All *S. suis* were kindly provided by the National Institute of Animal Health (NIAH) in Japan. Todd-Hewitt broth (THB) was used for the streptococcal culture. To investigate cross-reactivity, 5 strains of *S. pneumoniae* (TIGR4, EF3030, WU2, D39, and R6) were kindly provided by Dr. D.E. Briles, University of Alabama at Birmingham. The other strains were obtained from a research institute for microbial disease at Osaka University for this study (Table 1).

Table 1
Specificity of the ICS test.

Strain	Laboratory name	Serotypes	Source	Result of ICS (Relative intensity)
S. suis	10227	1	NIAH10227	1.45
	11318	1/2	NIAH11318	1.25
	31533	2	Diseased pig	1.85
	P1/7	2	Pig dying with meningitis	1.75
	90-1330	2	Healthy pig	1.91
	25018	2	Diseased human	1.90
	4961	3	Diseased pig	2.0
	6407	4	Diseased pig	1.64
	11538	5	Diseased pig	1.48
	2524	6	Diseased pig	1.4
	8074	7	Diseased pig	1.65
	14636	8	Diseased pig	1.8
	22083	9	Diseased pig	1.33
	13730	14	Diseased human	1.57
	2726	16	Diseased pig	1.45
S. agalactiae			RIMD 3114009	–
S. pneumoniae	R6	2	Human	–
	D39	2	Human	–
	WU2	3	Human	–
	TIGR4	4	Diseased human	–
	EF3030	19F	Human	–
S. aureus			ATCC 12598 Diseased human	–
E. faecalis			RIMD 3116001	–
K. pneumoniae			RIMD 1102003	–
E. coli	DH5-alpha			–
	EIEC		RIMD 0509763 Diseased human	–
P. aeruginosa			RIMD 1603001	–

2.2. Preparation of streptococcal antigen

To develop the immune antigens, streptococcal whole cells and CPS were selected. After *S. suis* was grown overnight in THB, the streptococcal broth was centrifuged at 8000 g for 10 min. The streptococcal pellet was washed 3 times in sterile phosphate-buffered saline (PBS) (Sigma-Aldrich, St. Louis, USA), and the washed cells were treated with 10% formalin for killing. Streptococcal CPS was removed for the immune antigen. The extraction and purification processes of CPS basically followed the procedure described in a previous report (Del Campo-Sepulveda et al., 1996), which is shown in Supporting document 1.

2.3. Confirmation of capsular polysaccharides

Confirmation of streptococcal CPS was conducted with native PAGE and western blotting as described previously (Tikkanen et al., 1995). Briefly, an 8% polyacrylamide slab gel was created in mini-Protean cells (Bio-Rad, Hercules, USA). Samples were mixed with a sample solution containing 0.65 M sucrose in 87 mM Tris-HCl, 4 mM EDTA, 1% bromophenol blue, with a pH of 8.0. Electrophoresis was conducted at 110 V for 90 min. After electrophoresis, the gel with CPS was dyed by adding 0.5% alcian blue. The CPS that was separated by the native PAGE was transferred from the slab gel to a polyvinylidene difluoride (PVDF) (Millipore, Billerica, USA) membrane using the methanol-Tris glycine method (Del Campo-Sepulveda et al., 1996). Electroblotting was performed in a transblot apparatus (Biorad, Hercules, USA) at 12 V for 90 min. Then, the PVDF membrane with CPS was blotted by the addition of Tris-buffered saline (25 mM Tris-HCl, pH 8.0, 2.7 mM KCl, 137 mM NaCl) containing 0.05% Tween 20 (TBST) with 3% skim milk, and the membrane was washed 3 times in TBST over the course of 1 h. After washing, some TBST was added to 0.05% *S. suis* type 2 antiserum (Statens serum institute, Hellerod, Denmark) and incubated for 1 h. The rest of the TBST was added to 0.05% rabbit IgG horseradish peroxidase (HRP) and incubated for 1 h, and then, the PVDF membrane was soaked in detection solution (GE Healthcare, Little Chalfont, UK) and exposed to the film.

2.4. Production of polyclonal antibodies

To obtain antibodies that would bind to *S. suis*, anti-*S. suis* polyclonal antibodies (pAbs) were produced. A female-specific, pathogen-free Kbs New Zealand white rabbit (1.5–1.99 kg) was obtained (Oriental Yeast, Osaka, Japan). After the rabbit was kept for 1 week, 1 ml of antigen solution that contained 500 μl of CPS (700 μg ml^{-1}) mixed with 500 μl of incomplete Freund's adjuvant (Becton Dickinson, Franklin Lakes, USA) was injected into the subcutaneous space using an 18 gauge needle and syringe (Terumo, Tokyo, Japan). The rabbit was immunised 3 times every 1 week. Immediately after administering the 4th immunisation, blood was sampled 4 days later. This rabbit serum was purified using a protein G column (GE Healthcare, Little Chalfont, UK). The concentration of the purified anti-*S. suis* antibodies was measured with a protein assay (Nacalai Tesque, Kyoto, Japan), and the affinity was confirmed by western blotting. The evaluation of antibody binding to CPS was conducted using an enzyme-linked immunosorbent assay (ELISA), and the method is shown in Supporting document 2.

2.5. Preparation of immunochromatographic strip test

The ICS test was prepared by the Alfresa Pharma Corporation (Osaka, Japan). The colloidal gold was used as the labelling particle, which had a tendency to increase the band clarity. The pAbs C-N1 and C-N2 were conjugated to colloidal gold, sprayed onto glass fibre pads, and then dried. The pAb C-N1 was micro-sprayed onto nitrocellulose membranes at 1.0 μl cm^{-1} at the position that would become the capture test line on the completed strips (Fig. 1). Rabbit