Rapid Screening of *Salmonella spp.* Using PBM BioSign™ Salmonella Test and Evaluation of the PBMS Test

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ABSTRACT : The PBM BioSign™ Salmonella (PBMS) test kit based on an immunochromatographic method was evaluated for the screening of *Salmonella spp.* in pure cultures, and 80, 15, and 10 artificially and naturally contaminated, and negative controlled food samples, respectively. The PBMS test involves presumptive qualitative procedures, *detecting* the presence of *Salmonella spp.* in foods within 26 h total testing period and allowing the user to release negative products 70 h earlier than the conventional methods. The PBMS test using Buffered Peptone Water and Rappaport-Vassiliadis broth was evaluated for 10 different food types for various *Salmonella spp.* It showed detection limits of 1 to 25 colony forming units (CFU)/25 g. No cross-reaction was observed, particularly to other gram-negative bacteria. These results indicate the PBMS test is a rapid and inexpensive procedure for the screening of *Salmonella spp.* present at low concentrations (1 to 25 CFU/25 g) in foods. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 12 : 1746-1750)***

Key Words : PBM BioSign™ Salmonella (PBMS) Test, Screening of *Salmonella spp.*

INTRODUCTION

The Center for Disease Control and Prevention (CDC) estimates that several million people in the United States become ill each year from eating food contaminated with harmful bacteria, such as *Salmonella* (Mead et al., 1999). Hazard analysis critical control point (HACCP) plans, and disinfection protocols may reduce direct horizontal transmission of *Salmonella spp.* However, control of *Salmonella* is difficult due to cyclic transmission between human hosts, animal hosts, and the environment, including feed (Williams, 1981). Pathogen reduction programs for *Salmonella* require rapid and efficient methods for monitoring the presence of these bacteria at appropriate points along the food chain. Therefore, accurate, sensitive, and rapid screening procedures for *Salmonella spp.* in contaminated foods, animal reservoirs, and humans are needed (Blackburn, 1993; Krusell and Skovgaard, 1993). Culture methods remain the standard method for the detection of *Salmonella spp.*; however, these procedures take 2 to 4 days to obtain presumptive results and up to 5 to 7 days for confirmatory results (Valdivieso-garcia et al., 2001). Current procedures include a pre-enrichment stage in a nonselective broth to allow the resuscitation and multiplication of sub-lethally damaged *Salmonella* organisms, an enrichment stage in selective broth to increase the ratio of *Salmonella* to competitor organisms, and an isolation stage using selective/differential agar media for the presumptive identification of any *Salmonella spp.* present. Demand from the food industry for reduction of testing costs and time to obtain results has led to the development of a rapid immunoassay for *Salmonella* detection (Flowers et al., 1989; Bird et al., 1999; Duncanson et al., 2003). The PBM BioSign™ Salmonella (PBMS) test is a presumptive qualitative test that detects the presence of *Salmonella spp.* in foods within 26 h, allowing the user to release negative products 70 h earlier than the conventional methods. Unlike classical methods for screening, which require tedious pipetting and washing steps to produce results, the PBMS test requires only one simple step. The objectives of this study were to determine the efficacy of the PBMS test for screening *Salmonella spp.* in foods and animal feeds and to quantify the detection limits of the assay.

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MATERIALS AND METHODS

Bacterial strains

In total, 10 different *Salmonella* serovars including major clinical serovars and 11 other pathogenic strains used in this study were listed in Table 1. The nomenclature of *Salmonella* follows the taxonomy described by Grimont et al. (2000). They were characterized using API 20E (bioMérieux, France).

Preparation of test organisms

All bacterial cells were grown in Tryptic Soy Broth (TSB, Difco, USA) for 18 to 24 h at 35 to 37°C. Pure cultures used for inoculation were stressed by storage at -20°C for 24 h before use. Food samples (25 g each) were individually inoculated with *Salmonella* stationary-phase cells in amounts sufficient for achieving levels of 1 to 25 and 50 to 200 colony-forming units (CFU)/25 g. Spread

Table 1. Bacterial strains, Minimum detection concentration and specificity of PBMS test

Organisms	Source	MDC [1]	Specificity
S. typhimurium (serogroup B)	ATCC 13311	1-10	+[3]
S. enteritidis (serogroup D1)	ATCC 34788	1-10	+
S. thomson (serogroup C1)	ATCC 8591	1-25	+
S. cholerasuis (serogroup C1)	ATCC 7001	1-16	+
S. typhi (serogroup D1)	ATCC 10749	1-15	+
S. paratyphi (serogroup A)	ATCC 33459	1-10	+
S. gallinarum (serogroup D1)	Isolated from chicken	1-12	+
S. pullorum (serogroup D1)	Isolated from chicken	1-10	+
S. tennessee (serogroup C1)	Isolated from feed	1-10	+
S. dublin (serogroup D1)	Isolated from feed	1-10	+
Escherichia coli	ATCC 43894	-	-[4]
Klebsiella pneumoniae	NVRQS[2]	-	-
Yersinia enterocolitica	NVRQS	-	-
Shigella sonnei	NVRQS	-	-
Enterobacter cloacae	NVRQS	-	-
Proteus mirabilis	NVRQS	-	-
Aeromonas caviae	NVRQS	-	-
Citrobacter freundii	NVRQS	-	-
Listeria monocytogenes	NVRQS	-	-
Staphylococcus aureus	FRI 913	-	-
Enterococcus faecium	ATCC 21912	-	-

[1] Minimum detection concentration (CFU/25 g) detected by PBMS test.
[2] National veterinary research and quarantine service in Korea.
[3] Positive result in PBMS test at a concentration of 3×10^5 CFU/ml.
[4] Negative result in PBMS test at a concentration of 3×10^8 CFU/ml.

plate counts were used to determine actual spike level of the original cultures.

Specificity

The specificity of the PBMS test kit was performed with 10 serovars of *Salmonella* and 11 non-*Salmonella* strains (Table 1). After growing in TSB overnight, the diluted cultures were tested using the PBMS test kits. The concentrations of *Salmonella* and non-*Salmonella* pure cultures used for the specificity test were 3×10^5 and 3×10^8 CFU/ml, respectively.

The PBMS test kit

The test device contains a dye pad impregnated with anti-*Salmonella* antibody-dye conjugate and a membrane strip, upon which anti-*Salmonella* antibody is immobilized in the test area. Appropriately enriched broth is added to the sample well of the PBMS test using a transfer pipette and allowed to soak into the pad. Any *Salmonella* antigens present in the specimen will react with the conjugate dye. This, in turn, migrates and binds to the immobilized antibody on the membrane in the test area, generating a colored band at the test position (T) in the result window. The result is read from 5 to 15 minutes; one line at the control position (C) indicates the absence of *Salmonella spp.*, signifying the test functioned properly. Two lines at C and T in the result window indicate the presence of *Salmonella spp.*

Artificially contaminated samples

Poultry, pork, mouse, and hamster feeds, chicken rinse water, powdered milk, liquid whole eggs, raw ground beef and pork, and fish (cod) meal were used for the matrix. Each food type was artificially inoculated with four different *Salmonella* serovars, which were selected randomly from 10 *Salmonella* serovars. One sample of each food type was left uninoculated as a negative control (Table 2). One set consisted of 80 artificially inoculated and 10 uninoculated food samples. For the evaluation, two sets were used. The first set was tested by the PBMS test and then confirmed by bacterial culture using selective agars, and biochemical and serological tests. The second set was performed by conventional method (Figure 1).

Naturally contaminated samples

Three, two, four, and six samples of chicken rinse water, feces of poultry, raw pork, and animal feeds, respectively, were tested with the PBMS test and consequent bacterial culture and were isolated following the conventional method as well.

PBMS test

Twenty-five grams of samples in the first set were separately added to 200 ml of pre-warmed Buffered Peptone Water (BPW, Difco) and mixed. The mixtures were incubated at 37°C for 6 to 8 h. After incubation, 200 ml Rapparport-Vassilidias (RV, Difco) broth was added to each

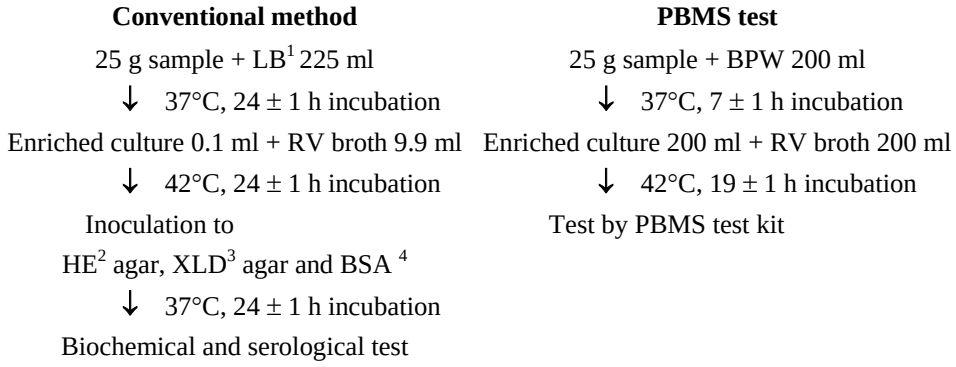

Figure 1. Procedures of conventional method and the PBMS test. The major differences between the two methods are the pre-enrichment incubation period and the amount and level of RV broth. [1] Lactose broth (LB, Difco), [2] Hektoen enteric (HE, Difco) agar, [3] Xylose lysine desoxycholate (XLD, Difco) agar, [4] Bismuth sulfate agar (BSA, Difco).

enriched culture, mixed, and incubated at 42°C for 18 to 20 h. Three drops of the enriched broth were added to the PBMS test kit. Results were read between 5 to 15 min. After testing, isolation of *Salmonella spp.* was performed using selective agars and biochemical tests in all samples. At the same time, isolations of *Salmonella spp.* in the second set of samples were performed using the conventional method (Figure 1).

RESULTS AND DISCUSSION

Specificity

The PBMS test detected all *Salmonella* serovars tested, which included representative serovars of serogroup A, B, C, and D, and gave negative results for all other bacteria species tested with 100% specificity (Table 1).

Sensitivity

The PBMS test was compared with the conventional method for artificially and naturally contaminated food sample groups. All samples were tested using the PBMS test and the conventional method. All naturally contaminated samples were found positive by both methods, showing 100% agreement (data not shown). Among the 80 artificially contaminated samples, 78, 77 and 76 samples showed positive results in the PBMS test, bacterial culture after the PBMS test, and the conventional method, respectively. All 10 uninoculated samples gave negative results for all test methods. Six of the artificially contaminated samples showed discrepant results between the PBMS tests and the conventional method. One sample was found positive with the PBMS test and bacterial culture after the PBMS test, whereas it was negative with the conventional method. Three were found positive by the PBMS test but negative with both bacterial cultures after PBMS tests and the conventional method. Two were negative with the PBMS tests but positive with bacterial cultures after PBMS tests and the conventional method (Table 2). Therefore, among all 80 artificially contaminated samples, the PBMS test showed 2.5% false negative rate (2 showed false negative among 80 artificially contaminated samples) and 0% false positive rate. The PBMS test showed the high sensitivity in spite of the reduced enrichment period.

Minimum detection concentration (MDC) of *Salmonella spp.*

MDCs of 10 different *Salmonella spp.* were determined as 1 to 25 CFU/25 g (Table 1). Thirty-six samples were determined to be between 1 to 10 CFU/25 g, and 4 samples were between 11 to 25 CFU/25 g (Table 2). The MDC of six samples, showing discrepancy in results among PBMS tests, bacterial cultures after PBMS tests, and conventional methods, were all between 1 to 10 CFU/25 g.

Salmonella, with more than 2,300 serovars identified, has persisted as one of the major foodborne pathogens (Doyle et al., 1997; Bae et al., 2003; Kang et al., 2003). Methods used for the detection of *Salmonella spp.* in food products and animal feeds involve a series of sequential culturing steps. In addition, pathogen contamination of foods often occurs at a very low level, less than one pathogen per gram of food. Several outbreaks of foodborne illnesses have resulted from the ingestion of small populations of injured pathogens in foods. Therefore, for the prevention of *Salmonella* infection, detection methods require not only rapidness and accuracy, but also capability to detect very low concentrations of organisms (Feng, 1995; Huang et al., 1999).

The PBMS test used in our study involved a decrease in incubation time and the inoculation of 200 ml of enriched whole BPW to 200 ml RV broth. We performed pre-experiments to establish the procedure of the PBMS test. Some different procedures before implementation of the PBMS test kit were tested. We adjusted the incubation time

Table 2. Analysis of artificially contaminated samples by PBMS test

Samples	*Salmonella* serovars	CFU /25 g	PBMS test[1]	Culture after PBMS test[2]	Conventional method	Samples	*Salmonella* serovars	CFU /25 g	PBMS test[1]	Culture after PBMS test[2]	Conventional method
Poultry feeds	*S. typhimurium*	7	+[3]	+	+	Powdered milk	*S. typhimurium*	7	+	+	+
		53	+	+	+			63	+	+	+
	S. enteritidis	8	+	+	+		*S. enteritidis*	8	+	+	+
		89	+	+	+			79	+	+	+
	S. gallinarum	8	+	+	NT[5]		*S. typhi*	3	+	-	-
		75	+	+	+			63	+	+	+
	S. tennessee	5	+	+	+		*S. thomson*	10	-	+	+
		64	+	+	+			128	+	+	+
	Negative control	0	-[4]	-	-		Negative control	0	-	-	-
Pork feeds	*S. typhimurium*	7	+	+	+	Liquid whole eggs	*S. typhimurium*	7	+	+	+
		83	+	+	+			63	+	+	+
	S. enteritidis	8	+	+	+		*S. typhi*	15	+	+	+
		59	+	+	+			98	+	+	+
	S. paratyphi	8	+	-	-		*S. pullorum*	5	+	+	+
		136	+	+	+			64	+	+	+
	S. tennessee	5	+	+	+		*S. enteritidis*	8	+	+	+
		74	+	+	+			59	+	+	+
	Negative control	0	-	-	-		Negative control	0	-	-	-
Mouse feeds	*S. typhimurium*	4	+	+	+	Raw ground beef	*S. typhimurium*	10	+	+	+
		63	+	+	+			72	+	+	+
	S. enteritidis	8	+	+	+		*S. enteritidis*	9	+	+	+
		79	+	+	+			100	+	+	+
	S. paratyphi	1	+	+	+		*S. typhi*	2	+	+	+
		57	+	+	+			113	+	+	+
	S. tennessee	5	+	+	+		*S. dublin*	8	-	+	+
		94	+	+	+			85	+	+	+
	Negative control	0	-	-	-		Negative control	0	-	-	-
Hamster feeds	*S. typhimurium*	7	+	+	+	Raw ground pork	*S. typhimurium*	7	+	+	+
		63	+	+	+			52	+	+	+
	S. enteritidis	2	+	+	+		*S. enteritidis*	9	+	+	+
		59	+	+	+			100	+	+	+
	S. dublin	8	+	+	-		*S. cholera suis*	16	+	+	+
		70	+	+	+			150	+	+	+
	S. tennessee	5	+	+	+		*S. thomson*	25	+	+	+
		74	+	+	+			128	+	+	+
	Negative control	0	-	-	-		Negative control	0	-	-	-
Chicken rinse water	*S. typhimurium*	6	+	+	+	Fish meal	*S. typhimurium*	3	+	+	+
		51	+	+	+			82	+	+	+
	S. enteritidis	2	+	+	+		*S. enteritidis*	2	+	+	+
		69	+	+	+			69	+	+	+
	S. thomson	9	+	+	+		*S. paratyphi*	9	+	+	+
		128	+	+	+			120	+	+	+
	S. gallinarum	12	+	+	+		*S. typhi*	2	+	-	-
		175	+	+	+			73	+	+	+
	Negative control	0	-	-	-		Negative control	0	-	-	-

[1] Results of PBMS test. [2] Results of bacterial culture after PBMS test.
[3] Positive. [4] Negative. [5] Not tested.

in BPW, the inoculation amount of BPW cultures, and the amount of RV broth. The procedure that yielded the best results was used for the PBMS test procedure (data not shown). The PBMS test was compared with a similar commercial screening method, Reveal for *Salmonella* test system (NEOGEN corp., USA) with 20 and 5 artificially contaminated and negative controlled samples, respectively. There was no significant difference between the two tests in terms of false-positive and false-negative rates ($p<0.05$, data not shown).

One hundred and five samples of various sources, including food and animal feed, were used to evaluate the

performance of the PBMS test. The results showed the positive rates of the PBMS test were higher than those of the conventional method. Previous studies reported that some commercial screening methods showed higher positive rates than the conventional culture method (Fierens and Huyghebaert, 1996). Among six samples showing discrepant results, the four matrixes were dried samples. The differences of the matrixes might have influenced the negative result in the conventional method. The *Salmonella* serovars of discrepant results were various; therefore it is uncertain whether there is an association between the *Salmonella* serovar and the detection rates of *Salmonella spp.*

This study showed the PBMS test was not only rapid (26 h total testing time) and accurate, showing high specificity and sensitivity, but also able to detect various *Salmonella spp.* at low concentrations (1 to 25 CFU/25 g). Results revealed that this test has potential for use in the implementation of pathogen reduction and HACCP systems as well as in clinical laboratories. Therefore, the PBMS test is applicable for the implementation of a practical and effective *Salmonella* reduction scheme.

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