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Reliability of the Immunodiffusion 1-2 Test™ System for Detection of *Salmonella* in Foods

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ABSTRACT

The novel 1-2 Test™ is a self-contained tetrathionate enrichment and immunodiffusion-agglutination reaction vial for the detection of *Salmonella* in preenrichment and direct enrichment cultures of processed and non-processed foods, respectively. Of 186 foods tested, 46 (24.7%) were found to contain salmonellae by all analytical conditions combined. The standard cultural procedure identified 43 (93.5%) contaminated samples whereas the 1-2 Test™ system detected 20 (43.5%) and 25 (54.3%) positive samples after 8 h and 24 h of incubation, respectively. Although six false-positive reactions were obtained after short (8 h) incubation of test vials, homologous reactions were not detected after 24 h incubation. System deficiency likely stems from the low selectivity of applied cultural conditions and inability of the 1-2 Test™ to detect salmonellae in the presence of large numbers of competing microflora.

The high sensitivity of standard cultural procedures for the detection of *Salmonella* in foods is overshadowed by the multiplicity of analytical steps and lengthy periods of time associated with the presumptive identification of positive samples *(3,14)*. Most attempts at method brevity have focused on enrichment broth cultures as test material thus offering an alternate approach to the more standard plating of enrichment cultures on differential agar media *(4)*. Immunological methods including the fluorescent antibody *(20)*, enrichment serology *(19)*, direct and indirect enzyme-linked immunosorbent assay (ELISA) procedures *(5,7,11,13,17)* have figured prominently in the search for rapid and sensitive diagnostic tools. The success of these technologies was recently challenged by the diagnostic potential of DNA probes arising from the hybridization of unique ^{32}p-labelled nucleotides with homologous *Salmonella* DNA in test samples *(10,12)*. More recently, the development of enzyme-conjugated (Vitek Systems, Hazelwood, MO) and chemiluminescent (MCLAS Technologies Inc., San Antonio, TX) phages for the detection of salmonellae in preenrichment cultures has generated great interest. However, the reliability of these innovative systems has yet to be evaluated collaboratively.

This report assesses the performance of the 1-2 Test™, a newly designed product for the identification of foodborne salmonellae in preenrichment and enrichment cultures through the formation of immunodiffusion bands in a semisolid agar medium.

MATERIALS AND METHODS

Food Analyses

The sensitivity of the *Salmonella* 1-2 Test™ with naturally contaminated foods (Table 1) was compared to a standard cultural procedure (Fig. 1). Dry foods were preenriched in nutrient broth (NB) overnight at 35°C, except chocolate which was suspended in reconstituted nonfat dry milk (10%) with added brilliant green *(15)*. Portions (1.0 ml) of preenrichment cultures were selectively enriched in 9 volumes of tetrathionate brilliant green (TBG_{43}) and selenite cystine (SC_{35}) broths incubated overnight at 43°C and 35°C, respectively. Concurrently, portions (0.1 ml) of preenrichment cultures were assayed by the 1-2 Test™ system.

High moisture foods were homogenized for 30 s to 60 s in a Waring blender with an equal volume of sterile water. Replicate 25 g-portions of slurries were directly enriched in TBG_{35} broth (direct TBG_{35}) and preenriched in NB. Following overnight incubation at 35°C, portions (0.1 ml) of direct TBG_{35} cultures were assayed by the 1-2 Test™ whereas NB cultures were selectively enriched in TBG_{43} and SC_{35} as previously described for dry foods (Fig. 1).

All selective enrichment cultures were plated on bismuth sulfite (BiS) and brilliant green sulfa (BGS) agar. Suspect colonies were screened biochemically on triple sugar iron (TSI) and lysine iron (LI) agar slants and presumptive *Salmonella* isolates confirmed serologically with polyvalent and single grouping antisera.

Salmonella 1-2 Test™

The immunodiffusion 1-2 Test™ (BioControl Systems Inc., Bothell, WA) is a two chambered plastic vial consisting of one inoculation and one motility chamber (Fig 2). The tetrathionate-brilliant green-serine broth in the inoculation chamber (chamber TBG_{35}) is first activated by the addition of iodine-iodide solution. A drop of antiserum preparation supplied by the manufacturer is then placed into the gel void located on the upper surface of the motility chamber. With the white cap securely fastened, the inoculation chamber plug is withdrawn to establish direct contact

TABLE 1. *Detection of* Salmonella *in foods.*

Food	Number of samples Tested	Positive (%)
High moisture		
Chicken		
Carcasses	15	9
Cut-up	12	3
Giblets	22	9
Nuggets	1	0
Turkey		
Giblets	3	0
Minced	1	0
Others[a]	3	0
Pork		
Minced	8	0
Sausages	22	3
Giblets	4	0
Others[b]	3	0
Beef/Veal		
Minced	8	1
Liver	2	1
Fermented sausage	4	0
Froglegs	1	1
Snail	2	2
Albumen (liquid)	1	0
Subtotal	112	29 (25.9)
Low moisture		
Albumen (dried)	2	0
Chocolate	8	1
Coconut	2	0
Dormane tea	1	0
Pasta	5	2
Spices		
Curry powder	1	0
Pepper (black)	17	3
Turmeric	1	1
Animal feeds	37	10
Subtotal	74	17 (23.0)
Total	186	46 (24.7)

[a]Turkey burgers (2) and sausage (1)
[b]Pork hocks (2) and stewing meat (1)

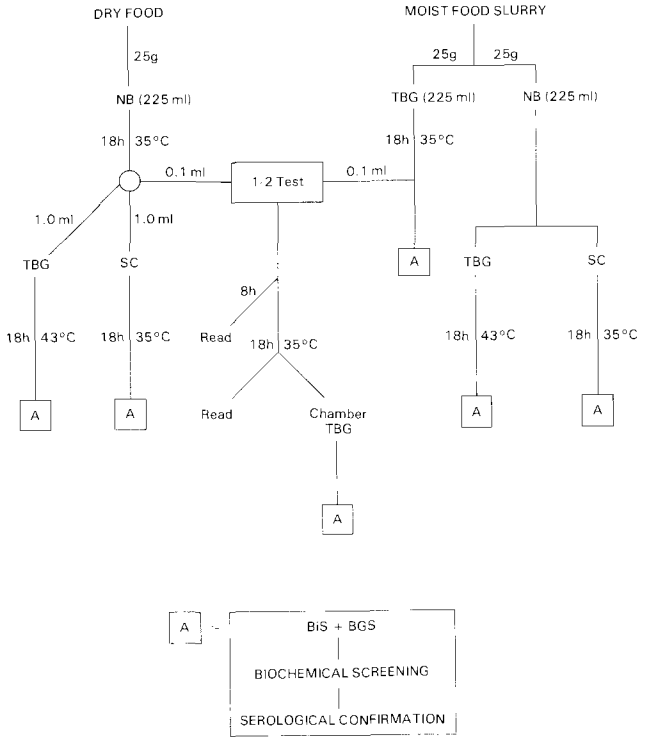

Figure 1. *Detection of foodborne* Salmonella *by the 1-2 Test™ and standard cultural procedures.*

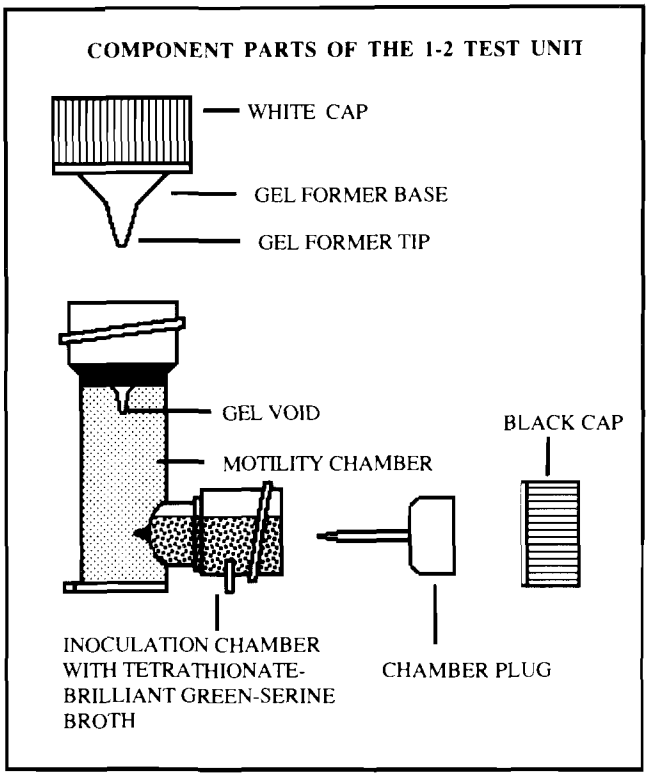

Figure 2. *Characteristics of the 1-2 Test™ vial.*

TABLE 2. *Performance of the* Salmonella *1-2 Test™ system*[a].

Food	Total samples		Number positive samples (%)		
	Tested	Positive	Cultural	1-2 Test™	
				8 h	24 h
High moisture	112	29	26(89.7)	13(44.8)	15(51.7)
Low moisture	74	17	17(100)	7(41.2)	10(58.8)
Total	186	46	43(93.5)	20(43.5)[b]	25(54.3)

[a]Data pertain to confirmed cultural and 1-2 Test™ results.
[b]Six additional false-positive reactions were obtained from high (3) and low (3) moisture foods.

TABLE 3. *Recovery of* Salmonella *at various stages of the 1-2 Test™ procedure.*

Food	Recovery pattern				Number of pattern reactions
	Direct TBG_{35}	Chamber TBG_{35}	1-2 Test (24 h)	Standard cultural	
High moisture (29)[a]					
	+	+	+	+	13
	+	+	+	-	2
	+	+	-	+	3
	-	+	-	+	2
	+	+	-	-	1
	-	-	-	+	8
Subtotal	19	21	15	26	29
Low moisture (17)[a]					
	NA[b]	+	+	+	10
	NA	+	-	+	7
Subtotal		17	10	17	17

[a]Total samples positive by combined cultural and 1-2 Test™ results.
[b]Not applicable.

between the inoculation and motility chambers. A portion (0.1 ml) of preenrichment or direct enrichment culture is then added to the chamber TBG_{35} broth. Inoculated vials are incubated at 35°C and checked for the formation of immunobands in the upper half of the motility chamber after 8 h and 24 h of incubation. A white line of precipitation results from an antibody-dependent immobilization of motile salmonellae in the semisolid medium. Positive and negative control reactions with *Salmonella typhimurium* and *Enterobacter cloacae* were also run concurrently with test samples.

RESULTS AND DISCUSSION

Of 186 foods tested, 46 (24.7%) were found to contain *Salmonella* by either culture or immunodiffusion techniques (Table 1). Our choice of NB rather than the lactose broth recommended by the manufacturer for preenrichment would not adversely affect the performance of the 1-2 Test™ system because earlier work clearly established the equivalence of both non-selective media *(6)*. Three positive poultry samples were not detected by the conventional cultural procedure (Table 2). Of these, two chicken carcasses were recognized by the 1-2 Test™ after 24 h incubation whereas only one of these two samples was identified after short (8 h) incubation. A sample of chicken liver was also found to be negative by the cultural and completed 1-2 Test™ but yielded salmonellae in the direct TBG_{35} and chamber TBG_{35} cultures (data not shown). Such inability of standard cultural methods to detect *Salmonella* in foods has been noted previously *(8,12)*. Early (8 h) examination of the 1-2 Test™ vials identified fewer than 50% of contaminated samples with some increase in sensitivity after 24 h of incubation (Table 2). False-positive results were obtained after 8 h but not 24 h of incubation. Such aberrant reactions were equally distributed between low and high moisture foods. In addition to the typical positive reaction of an inverted bell-shaped immunoband, other positive reactions such as faint, asymmetric and double immunobands were encountered on several occasions. The latter occurrence likely resulted from development of both flagellar phases of *Salmonella* in the inoculation chamber. Single and double immunobands just above the opening of the inoculation chamber into the semisolid agar medium were also encountered and read as negative reactions.

Insight into the noted deficiencies of the system was obtained through concurrent plating of direct TBG_{35} and chamber TBG_{35} cultures (Table 3). Direct enrichment of meats and other high moisture foods identified only 19/26 (73.1%) samples found to be contaminated by the standard cultural procedure. Inability of direct enrichment to reliably provide contaminated culture material therefore compromised at the outset the efficacy of the 1-2 Test™ system. These findings underline the merits of preenrichment for the recovery of *Salmonella* in all food types irrespective of their incident levels of background flora *(1,4,14)*. The greater selectivity of enrichment media incubated at elevated temperatures (≥41°C) is well established *(2,3,9,16,18)*. This factor likely played in the greater number of isolations by the standard TBG_{43} than by the direct TBG_{35} enrichment

method. Although chamber TBG_{35} cultures potentiated the identification of 21 high moisture and 17 low moisture foods, the completed 1-2 Test™ identified only 15 and 10 of these samples, respectively (Table 3). Conceivably, the presence of overwhelming numbers of non-salmonellae in the chamber TBG_{35} arising from the enrichment of foods at a permissive temperature (35°C) and in small volumes (1.2 ml) of chamber TBG_{35} broth hindered agglutination reactions between motile *Salmonella* and diffusing antibody.

Sensitivity of the 1-2 Test™ seemingly could be increased through non-selective enrichment (preenrichment) of all food samples followed by selective enrichment in TBG_{43} for 18 h to 24 h prior to inoculation into 1-2 Test™ vials. Enrichment in a second selective medium such as selenite-cystine, RV *(21)* or other suitable broth medium together with TBG_{43} would likely increase method sensitivity. Such an approach would not only insure resuscitation of the few stressed or injured *Salmonella* likely to contaminate food samples but also provide for a facilitated detection of the pathogen through establishment of more favorable ratios of *Salmonella* to competitive flora. Indications that the proposed change in analytical protocol is sound can be seen in recent changes in the manufacturer's package inserts requiring overnight preenrichment and selective enrichment of animal feeds and flour-based products in TBG_{35} for 24 ± 2 h (BioControl Systems Inc., 1987). Further studies on optimal conditions for the reliable detection of foodborne *Salmonella* by the 1-2 Test™ are clearly indicated.

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