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Rapid detection of *Salmonella* in foods using a combination of SPRINT™, MSRV™ and Salmonella Latex Test™

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A new procedure for rapid detection of Salmonella in foods, based on the combination of SPRINT™, MSRV™ and Salmonella Latex Test™, was evaluated. SPRINT™ is a system to reduce the pre-enrichment and selective enrichment steps to 24 hours. MSRV™ is a semi-solid selective media for detection of motile Salmonella. Salmonella Latex Test™ is a rapid latex agglutination test for Salmonella. Using the three systems in combination, the total time for detection of Salmonella in a food sample is 48h. Evaluations were performed in artificially contaminated ready-to-eat baby-foods and raw Brazilian sausages (lingüiça) containing no added microorganisms. The BAM conventional culture procedure was used as reference method. The study with baby foods indicated that the new procedure had good sensitivity (89%) and specificity (100%), without cross-reactions with Enterobacteriaceae. However, when applied to naturally contaminated foods, the performance was poor: chi square (χ^2 = 5.062, $\alpha \geq$ 0. 05) and Kappa-Cohen agreement (K = 0.171, p=0.089) indexes indicated that the differences between results given by the two procedures were significant and the correlation between them was low.

Uniterms:
- *Salmonella*
- Food microbiology
- SPRINT™
- MSRV™
- Salmonella Latex Test™

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INTRODUCTION

Salmonella is one of the most important pathogens that challenge the food industry today. This pathogen remains one of the leading causes of human foodborne infections in most countries. In Brazil, the Pan American Health Organization, from WHO, reported that *Salmonella* (including *S.* enteritidis and *S.* typhi) was the most frequent agent, being responsible for 58.1% of the outbreaks due to bacteria, and 66.2% of the cases with known etiology occurred between 1995 and 2001 (Franco *et al.*, 2002). Aside from the appalling human cost, outbreaks associated with a food product also bring a significant financial burden.

The number of samples tested for *Salmonella* increases constantly as a result of enhanced surveillance by both the food industry and governmental health authorities. The conventional standard cultural method for detection of *Salmonella* in foods is labor-intensive, costly and time-consuming. A complete test requires several

culture media, a considerable amount of labware and a lot of work. Three days are required for a negative result, while confirmed positive results become available only after five days (Andrews, Hammack, 2001; Andrews *et al*., 2001).

Over the years, a number of new rapid *Salmonella* screening tests have been proposed (Fung, 1995; Franco, 1999; Feng, 2001). The Bacteriological Analytical Manual, from the U.S. Food and Drug Administration, describes 25 antibody-based and four nucleic acid-based commercially available assays for rapid detection of foodborne *Salmonella*, plus 18 miniaturized kits for identification of this pathogen (Feng, 2001). Despite the great number, only a few are routinely used in food microbiology laboratories, due to limitations of their application.

Two of them, the semi-solid Rappaport-Vassiliadis medium (MSRV) and the Oxoid Salmonella Latex Text, are widely used and particularly useful to reduce time and labor in the laboratory (De Smedt *et al.*, 1986; De Smedt, Bolderdijk, 1987; Holbrook *et al.*, 1989. Poppe, Ducan, 1996; O´Donogue, Winn, 1993; De Smedt *et al.*, 1994; Dusch, Altwegg, 1995; Wiberg, Norberg, 1996; De Zutter, Arnaut-Rollier, 1999; Worcman-Barninka *et al.*, 2001).

The MSRV medium is a semi-solid medium for detection of motile *Salmonella* from food and environmental samples and is an official AOAC method (Feng, 2001). It contains selective agents (malachite green oxalate, magnesium chloride and novobiocin) and 2.7% agar. The efficiency of this medium is based on the ability of *Salmonella* to migrate through the selective medium ahead of competing motile microorganisms, thus producing opaque halos of growth (Oxoid, 1998).

The Oxoid Salmonella Latex Test is an agglutination test for the presumptive identification of *Salmonella* isolated by the Oxoid Salmonella Rapid Test. This kit is based on latex particles sensitized with polyvalent *Salmonella* antibodies (rabbit IgG) (Oxoid, 1998). In theory, this test can be used for presumptive identification of *Salmonella* isolated by any other methodology. Identity should be confirmed by standard biochemical or serological techniques.

The novel SPRINT (Simple Pre-enrichment and Rapid Isolation Novel Technology) technology was recently introduced in the Brazilian market. The aim of SPRINT is the reduction of time required for the pre-enrichment and selective enrichment steps. This system comprises three components: an enrichment broth, containing specially designed peptones for fast recovery of stressed *Salmonella*, a recovery supplement containing Oxyrase™, and timed-release capsules that when added to the broth burst after 6 hours and release the content turning the medium fully selective for *Salmonella*. At the moment, very few reports on the performance of this new *Salmonella* enrichment procedure are available (De Zutter, Arnaut-Rollier, 1999; Richter *et al.*, 2000).

Despite the existence of these three rapid systems for *Salmonella* their combined use in foods was not reported yet. Thus, the objective of this study was to evaluate the performance of the combination *between* SPRINT, MSRV and Salmonella Latex Test systems for rapid detection of *Salmonella* in foods. Evaluations were performed in three steps: 1. in ready-to-eat baby-foods, artificially contaminated exclusively with *Salmonella*; 2. in ready-to-eat baby-foods, artificially contaminated with *Salmonella* plus a cocktail of competing Enterobacteriaceae; 3. raw Brazilian sausages (lingüiça), purchased in local stores in São Paulo, without artificial addition of microorganisms. The conventional standard cultural method, as recommended by Andrews, Hammack (2001), for foods with high microbial load, was used as reference method.

MATERIALS AND METHODS

Microorganisms. *Salmonella* Enteritidis and a cocktail of Enterobacteriaceae, composed by strains of *Escherichia coli, Citrobacter freundii* and *Proteus mirabilis*, isolated from foods in the Food Microbiology Laboratory of Faculty of Pharmaceutical Sciences of University of Sao Paulo, Sao Paulo, Brazil.

Foods. Ready-to-eat shelf-stable baby-foods (150 g/flask), and refrigerated raw Brazilian sausages (lingüiça), containing chicken or pork ground meat, purchased in local stores prior to their expiration date. Refrigerated lingüiça samples were transported to the laboratory in cooled containers and tested within two hours.

Pre-enrichment in SPRINT. 25 g of food sample was stomached (Seward Medical Ltd. London, UK) with 225 mL of Salmonella Enrichment Broth (Oxoid CM966B), supplemented with 2 mL of Salmonella Recovery Supplement (Oxoid SR179A). After stomaching, six units of SPRINT Salmonella Timed-Release Capsules (Oxoid SR180A) were added. The mixture was incubated at 42 °C for 24 ± 1 h, with gentle shaking. (Figure 1).

Plating on MSRV agar. At the end of the incubation of SPRINT, 0.1 mL of the broth was spotted on the surface of a plate containing 12-15 mL of MSRV medium (Oxoid

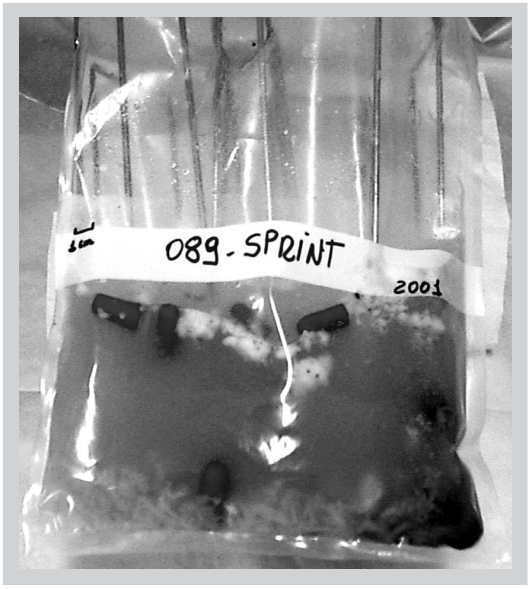

FIGURE 1 - The SPRINT system, showing the bursting of the timed-released capsules after 6 h of incubation.

CM910), using a sterile pipette. Plates were incubated in upright position at 42 °C for 18-24 h. Plates were checked for a migration zone (white halo, with radius larger than 10 mm) around the spot (Figure 2).

Confirmation of *Salmonella.* The bacterial growth at the edges of the migration zones were transferred to Tryptone Soya Broth (Oxoid CM129), incubated at 35 °C for 1-2 h under gentle agitation and submitted to agglutination tests using Salmonella Latex Text (Oxoid FT203). An aliquot of the broth was transferred to the surface of the reaction

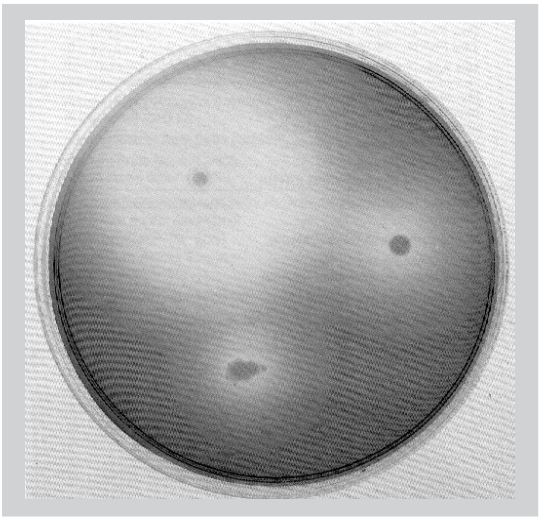

FIGURE 2 - The MSRV agar, showing the halos of motile *Salmonella*.

card, and homogenized with the latex reagent by means of a sterile rod. Agglutination of latex particles was considered a presumptive positive result for *Salmonella* (Figure 3). Absence of agglutination was interpreted as absence of *Salmonella*.

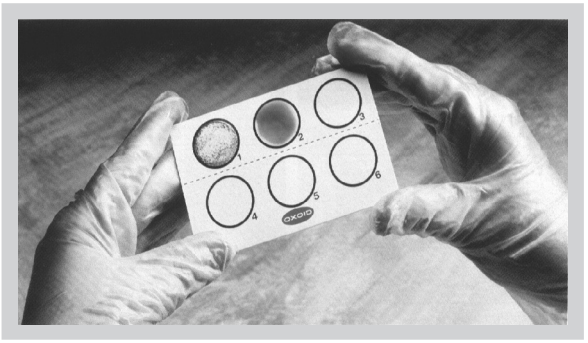

FIGURE 3 - Salmonella Latex Test, showing agglutination.

Confirmation of a presumptive positive result for *Salmonella*. All presumptive positive results for *Salmonella* in Salmonella Latex Text were confirmed transferring part of the growth at the edges of the migration zones in MSRV to the surface of plates containing Tryptone Soy Agar (Oxoid CM131) and incubated at 35 °C for 18-24 h. Isolated colonies were transferred to tubes with Triple Sugar Iron agar (Oxoid CM277), and Lysine Iron agar (Oxoid CM381) and incubated at 35 °C for 18-24 h. When necessary, additional biochemical tests were performed, using the API-20E (bioMérieux) system. Cultures identified as *Salmonella* were submitted to serological tests using polyvalent antisera containing somatic and flagellar antibodies (Probac do Brasil Produtos Bacteriológicos Ltda).

Conventional standard cultural method (Andrews, Hammack, 2001). 25 g of the sample were homogenized with 225 mL Lactose Broth (Oxoid CM137), using a stomacher (Seward Medical Ltd. London, UK). The mixture was incubated at 35 °C for 24 h. Then 1.0 mL of this broth was transferred to Tetrathionate Broth (Oxoid CM029) and 0.1 mL was transferred to Rappaport-Vassiliadis broth (Oxoid CM669A), incubated for 24 h at 35 °C and 43 °C, respectively. Aliquots of these broths were streaked on the surface of plates containing Bismuth Sulphite agar (Oxoid CM201), XLD agar (Oxoid CM469), Hektoen Enteric Agar (Oxoid CM419) as recommended by Andrews, Hammack, 2001, and also in MLCB agar (Oxoid CM783). All plates were incubated at 35 °C for 24 h. Three suspect colonies (presumptive positive *Salmonella*) from

each agar were submitted to complete identification as described above.

Serotyping of *Salmonella*. All *Salmonella* strains isolated from refrigerated raw Brazilian sausages (lingüiça), regardless the isolation method, were submitted to complete serotyping at the Enterobacteriaceae Reference Laboratory of Adolfo Lutz Institute, Sao Paulo, Brazil.

Evaluation in artificially contaminated foods. Samples of baby-foods were artificially contaminated with *Salmonella* Enteritidis exclusively, in order to get 5, 10, 10^2, 10^3, 10^4, 10^5 or 10^6 CFU/g. Samples were also contaminated with *S*. Enteritidis plus a cocktail of competing Enterobacteriaceae, in order to achieve 5, 10, 10^2 and 10^3 CFU of *S.* Enteritidis and 10^3 and 10^6 CFU of Enterobacteriaceae per gram of product. For contamination, cultures of microorganisms in logarithmic growth phase in Brain Heart Infusion broth (Oxoid CM225) were used. The exact number of inoculated microorganisms was determined by plating decimal serial dilutions of the BHI broth in BHI agar plates. Before inoculation, an aliquot of each baby-food sample was withdrawn for investigation of *Salmonella,* using the standard culture method. After addition of cultures, samples were homogenized in a stomacher for 30 seconds at low speed. Twenty-five grams of each baby-food sample (inoculated and non-inoculated controls) were tested for *Salmonella* using the proposed new method and the conventional standard culture method simultaneously. Each experiment was repeated five times. Table 1 summarizes the levels of contamination with *Salmonella* and Enterobacteriaceae used in the experiments.

TABLE I - Levels of contamination of baby-foods with *Salmonella* and Enterobacteriaceae

Experiment #	*S.* Enteritidis (CFU/g)	Cocktail of Enterobacteriaceae* (CFU/g)
1	5	10^3
2	10	10^3
3	10^2	10^3
4	10^3	10^3
5	5	10^6
6	10	10^6
7	10^2	10^6
8	10^3	10^6

**Escherichia coli*, *Citrobacter freundii* and *Proteus mirabilis*

Evaluation in naturally contaminated foods. One hundred samples of refrigerated raw Brazilian sausages (lingüiça) were tested. Each sample was homogenized with equal weight of sterile saline, using a stomacher. One 25 g portion of this homogenate was homogenized with 225 mL of SPRINT broth and a second portion of 25 g was homogenized with 225 mL of Lactose Broth. The first broth was used for investigation of *Salmonella* by the method under evaluation and the second by the conventional standard culture method. Only those samples in which the presence of *Salmonella* was confirmed by complete serotyping at Instituto Adolfo Lutz were considered as *Salmonella* positive. For a better evaluation of the SPRINT system, SPRINT broths were also streaked onto agar plates used in the conventional standard culture method.

Statistical Analysis. Sensitivity and specificity of the proposed method were evaluated according to Boer, Beumer, 1999, i.e.

$$\text{Sensitivity (\%)} = \frac{\text{number of true positives (p)} \times 100}{\text{p + number of false-negatives}}$$

$$\text{Specificity (\%)} = \frac{\text{number of true negatives (n)} \times 100}{\text{n + number of false-positives}}$$

Results for *Salmonella* in naturally contaminated foods were submitted to statistical analysis using McNemar test (Siegel, Castellan, 1988). A c^2 value superior to 3.84 indicated a significant difference for a = 0.05. The Kappa index was determined to evaluate the degree of agreement between results achieved by the two methods (Roitman, Boice, 1982).

RESULTS AND DISCUSSION

Evaluation in artificially contaminated foods

The proposed method, based on the combination of SPRINT™, MSRV™, and Salmonella Latex Test™ was able to detect the presence of *Salmonella* in artificially contaminated baby-foods when the level of the pathogen was as low as 10 CFU/g, regardless the presence of competing Enterobacteriaceae. In those samples where the level of contamination was only 5 CFU/g, results for *Salmonella* were negative. Non-inoculated baby-food samples were also negative. Table 2 presents the results achieved by the two methods in relation to the detection of *Salmonella* in the presence of other microorganisms.

TABLE II - Positivity for *Salmonella* Enteritidis in baby-foods artificially contaminated with this pathogen and with a cocktail of Enterobacteriaceae

S. Enteritidis (CFU/g)	cocktail of Enterobacteriaceae (CFU/g)	Standard culture method	Proposed method
0	10^3	negative	negative
0	10^6	negative	negative
5	10^3	negative	negative
5	10^6	negative	negative
10	10^3	negative	positive
10	10^6	negative	positive
10^2	10^3	positive	positive
10^2	10^6	positive	positive
10^3	10^3	positive	positive
10^3	10^6	positive	positive

The proposed method was more effective than the conventional standard cultural method to detect low concentrations of *Salmonella* in the inoculated samples. When the amount of *Salmonella* in the food was 10 CFU/g, the conventional standard cultural method failed to detect the pathogen, even when competing Enterobacteriaceae were not present. For higher concentration (> 10^2 CFU/g) both methods performed equally well, regardless the amount of Enterobacteriaceae in the sample.

Based on the number of positive and negative results achieved in the five repetitions for each level of *Salmonella* and Enterobacteriaceae in the product, the calculated sensitivity for the proposed method was 89%. No false-positive result was observed, thus the specificity was 100%.

Evaluation in naturally contaminated foods

Salmonella was detected in 17 out of 100 samples of Brazilian sausages (lingüiça). Their serotypes are presented in Table 3. The positivity for *Salmonella* according to detection method is presented in Table 4.

As shown, among 17 *Salmonella* positive samples, 14 were positive by the conventional standard cultural method and four by the proposed method. Only one sample resulted *Salmonella* positive by both methods, simultaneously. χ^2 value for these results was 5.062 ($\alpha \geq 0{,}05$) and the Kappa correlation index was 0.171 ($p=0.089$), which indicate that differences among results were significant. In addition, the Kappa correlation index was low, indicating poor agreement between results of the tests.

TABLE III - Serotypes of *Salmonella* detected in Brazilian sausages (lingüiça)

Serotypes	Number os samples
Salmonella Panama	6
Salmonella Typhimurium	5
Salmonella Enteritidis	5
Salmonella Derby	1
Total	17

TABLE IV - Positivity for *Salmonella* in Brazilian sausages (lingüiça) using the proposed method and the conventional standard cultural method.

Results	Number
Tested samples	100
Salmonella positive samples by one method at least	17
Salmonella positive samples by the standard cultural method	14
Salmonella positive samples by the proposed method	
Presumptive positive	9
Confirmed positive	4
Salmonella positive samples (confirmed) by the standard cultural method and by the proposed method simultaneously	1

It should be pointed out that nine samples presented presumptive positive results for the proposed method, i.e. positive results given by the latex agglutination test, prior to complete serotyping. The occurrence of only four confirmed results among nine presumptive positive results can be taken as a disadvantage of the proposed method. There is a general agreement that rapid methods are for screening only, and positive results should be considered presumptive, requiring confirmation by means of a standard cultural method (Feng, 2001).

Another important point is that complete serotyping is a procedure rarely used in food quality control laboratories, except when elucidation of salmonelosis outbreaks and/or cases is needed. Thus, routine laboratory testing should rely on other strategies to confirm the positive results obtained by the rapid methods. However, the confirmation of positive results by the standard cultural method, despite universally recommended, is not always completely reliable. In our study, three *Salmonella* positive sausage samples, as detected by the proposed method and confirmed by complete serotyping, were negative by the standard cultural method.

In order to detect which one of the three systems used in the proposed method could be the responsible for the lower positivity for *Salmonella* than that achieved by the reference method, the SPRINT enrichment broth was also plated in the selective agar plates used in the standard cultural method. When SPRINT was plated on the selective agars instead of MSRV medium, the number of *Salmonella* positive samples increased to nine, but remained lower than that achieved by the reference method (14 positive samples). χ^2 value for these results was 1.066 ($\alpha \geq 0.05$) which indicates that differences among them were not significant. However, the Kappa correlation index (0.678, p=0.091) showed only partial agreement between results. These indexes indicate that the failure in detecting all *Samonella* positive samples by the proposed method can be charged to the use of MSRV for plating and Salmonella Latex Test for confirmation of *Salmonella* positive samples. Both systems had already been demonstrated to be very sensitive to detect *Salmonella* (De Smedt *et al.*, 1986; De Smed, Bolderdijk, 1987; Holbrook *et al.*, 1989; De Zutter, Arnaut-Rollier, 1999; Poppe, Ducan, 1996; O´Donogue, Winn, 1993; De Smedt *et al.*, 1994; Dusch, Altwegg, 1995; Wiberg, Norberg, 1996; Worcman-Barninka *et al.*, 2001), but our results indicate that their combined use cannot be recommended.

In conclusion, the proposed method based on the combination of SPRINT™, MSRV™ and Salmonella Latex Test™ did not perform well for detection of *Salmonella* in naturally contaminated Brazilian raw sausages (lingüiça), despite its excellent performance in artificially contaminated foods (baby-foods), containing different concentrations of *Salmonella* and competing Enterobacteriaceae. This leads to conclude that chemicals or other microorganisms in the food matrix, low pH and natural or added components in the food, or extrinsic parameters such as low temperature, interfere in the performance of the proposed detection method. Further testing, with other types of foods, are still needed for a better evaluation of the performance of this procedure for rapid detection of *Salmonella* in foods.

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RESUMO

Detecção rápida de *Salmonella* em alimentos empregando uma combinação de SPRINT®, MSRV® e Salmonella Latex Test®

Avaliou-se um novo procedimento para detecção rápida de Salmonella em alimentos, baseado na combinação entre SPRINT®, MSRV® e Salmonella Latex Test® . SPRINT® é um sistema para reduzir as etapas de pré-enriquecimento e enriquecimento seletivo para 24 h. MSRV® é um meio seletivo semi-sólido para detecção de salmonelas móveis. Salmonella Latex Test® é um teste rápido de aglutinação de látex. A combinação dos três sistemas permite que a detecção de Salmonella em alimentos possa ser feita em apenas 48 h. O procedimento foi avaliado em alimentos infantis prontos para consumo, experimentalmente contaminados com Salmonella exclusivamente e com uma mistura de Salmonella e várias espécies de Enterobacteriaceae e também em cem amostras de lingüiças de porco e de frango sem adição artificial de microrganismos. O método convencional de cultura foi empregado como método de referência. A avaliação em alimentos infantis indicou que o procedimento proposto apresentava boa sensibilidade (80%) e especificidade (100%), sem reação cruzada com outras Enterobacteriaceae. Entretanto, quando aplicado a lingüiças, seu desempenho não foi adequado: os valores de χ^2 (5,062, $\alpha \geq 0,05$) e do índice de concordância de Kappa (0,171, p=0,089) indicaram que as diferenças entre os resultados obtidos pelos dois métodos foram estatisticamente significantes e a correlação entre eles foi baixa.

UNITERMOS: Salmonella. *Alimentos*. *SPRINT™*. *MSRV™*. *Salmonella Latex Test™*.

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