Evaluation of the Compact Dry SL method for the detection of *Salmonella* in spiked food samples.

Ellis P*, Kirchhof G# and Meldrum R*

* NPHS for Wales, Llandough Hospital, Penarth, CF64 2XX

Scil Diagnostics GmbH, Fraunhoferstrasse 15, D-82152 Martinsried, Germany

Introduction

Salmonella continues to be one of the main causes of human food borne infection, with between 16,000 and 27,000 cases reported annually in the UK over the last decade. The primary reservoirs for *Salmonella* are the gastrointestinal tracts of both, wild and domestic animals, and birds, particularly poultry. Transmission occurs predominantly via foodstuffs such as meat, raw eggs, milk and dairy products, which have been insufficiently cooked or have been contaminated, post cooking.

The traditional method for the detection of *Salmonella* in foods requires four successive stages, namely pre-enrichment in a non-selective media, enrichment in selective broth, subculture to selective agar and presumptive identification, and finally confirmation of presumptive colonies using biochemical and serological identification.

The use of dry film methods for specific pathogens are a relatively recent innovation in methodology and are aimed at providing laboratories with an alternative to traditional methods, by offering a method that uses dehydrated selective or differential media in a novel format.

Poster presentation at HPA 1st Scientific Conference, University of Warwick, September 2003.

The purpose of this study was to evaluate one such method, the Compact Dry SL method for detecting the presence of *Salmonella*. Forty spiked ready-to-eat foods were tested using the Compact Dry SL method and standard PHLS

Method

An overnight culture of *Salmonella* was prepared in nutrient broth, from which serial dilutions were prepared and inoculated onto blood agar plates, to determine spiking levels.

A 1:10 dilution of each ready-to-eat food was then prepared by weighing 25 g of food into a sterile homogeniser bag, adding 225 ml of buffered peptone water, and homogenising in a stomacher for 30 seconds.

Each homogenate was then spiked with between 2 and 26 cfu/25 g of food, and incubated at 37^{o}C for 16-18 hours.

Following incubation, 1 ml of the homogenate was inoculated into Selenite Cystine broth, which was then incubated at 37^{0}C for 18-24 hours, and 0.1 ml of the homogenate was inoculated into Rappaport Vassiliadis broth, which was then incubated at 42^{0}C for 18-24 hours.

Following incubation, the Selenite Cystine broth and Rappaport Vassiliadis broth were each inoculated onto a Brilliant Green agar plate and a Desoxycholate agar plate. All plates were then incubated at 37^{0}C for 18-24 hours.

The Compact Dry SL plate was also inoculated from the incubated buffered peptone water. 0.1 ml of the homogenate was placed onto the surface of the plate approximately 1 cm from the bottom edge. 1 ml of sterile water was then inoculated onto the surface of the plate at the opposite point to that of the

homogenate (Figure 1). The Compact Dry plates were then incubated at 42^0C for 24 hours

Following incubation, all plates were examined for suspect *Salmonella* colonies. All suspect colonies were then subcultured on MacConkey agar and confirmed as *Salmonella* by serological and biochemical (API) testing.

Results

Sample	Estimated Spike Cfu/25 g	*Salmonella* result	
		Compact Dry SL	PHLS Standard method
Chicken	6	Positive	Positive
Sausage	6	Positive	Positive
Beef	6	Positive	Positive
Beef	6	Positive	Positive
Beef	6	Positive	Positive
Ham	6	Positive	Positive
Ham	6	Positive	Positive
Ham	6	Positive	Positive
Milk	26	Positive	Positive
Milk	26	Positive	Positive
Milk	26	Positive	Positive
Milk	26	Positive	Positive
Milk	2	Positive	Positive
Milk	2	Positive	Positive
Milk	2	Positive	Positive
Milk	2	Positive	Positive
Meat pie	2	Positive	**Negative**

Meat pie	2	Positive	Positive
Meat pie	2	Positive	Positive
Meat pie	2	Positive	Positive
Meat pie	2	Positive	Positive
Meat pie	2	Positive	Positive
Meat pie	2	Positive	**Negative**
Meat pie	2	Positive	Positive
Ice cream	3	Positive	Positive
Ice cream	3	Positive	Positive
Ice cream	3	Positive	Positive
Ice cream	3	Positive	Positive
Ice cream	3	Positive	Positive
Ice cream	3	Positive	Positive
Ice cream	3	Positive	**Negative**
Ice cream	3	Positive	Positive
Burger	3	Positive	Positive
Bacon roll	3	Positive	Positive
Chicken s'wich	3	**Negative**	Positive
Ham salad s'wich	3	Positive	Positive
Burger	3	Positive	Positive
Ham s'wich	3	Positive	Positive
Beef s'wich	3	Positive	Positive
Chicken roll	3	**Negative**	Positive

Discussion

Recovery rates were 95% with Compact Dry SL and 93% with the PHLS standard method. The samples where *Salmonella* was not recovered had been spiked with low numbers (2-3 cfu/25 g).

The Compact dry SL plates were easy to read, and a high degree of selectivity was noted. Negative plates remained a light blue colour (Figure 2), whilst presumptive *Salmonella* growth appeared as black or yellow coloured areas or isolated colonies (Figure 3), or when heavy growth occurred, no colonies were visible but the entire plate became yellow (Figure 4). Coliform organisms display a blue–red/purple colour in the media, but were seldom present, unlike the growth obtained using the standard method, where the presence of lactose fermenting organisms were observed with all samples tested. Subculturing of suspect colony growth, for confirmation testing, was straightforward with both methods. The PHLS standard method produced typical colonies on the surface of the selective agar plates, which were easy to pick. The Compact Dry method seldom gave discrete colonies as growth mainly occurred within the gel matrix of the dry film plate and not on the surface. Therefore, areas of the plate where a colour change had occurred were swept with a loop and then streaked onto MacConkey agar for isolation of single colonies. Subsequent confirmation was identical for both methods. Serological and biochemical tests were performed from the subcultures on MacConkey agar.

The Compact Dry SL method was more rapid than the standard method, a negative result was available after 48 hours compared with 72 hours for the

standard method, confirmed positive results were available after four days. Other advantages of the Compact Dry SL method was, that less waste is generated, as it is a single plate test, compared to the standard method which requires the use of two selective broths and four selective agar plates. The Compact Dry SL method is also, therefore a much less labour intensive method.

Conclusion

The Compact Dry SL method was considered to be suitably comparable to the standard method in terms of detection, with additional advantages such as waste and labour reduction, easier colony identification and a more rapid confirmation of negative and positive results.