

Detection of Very Low Levels of Stressed *Salmonella* in Food in 10½ hours with the Alaska AK-phage[®] Method.

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ABSTRACT

A novel rapid microbiological method has been developed for the detection of *Salmonella* in food within 10½ hours. Derived from UK Ministry of Defence battlefield detection technology, the Alaska AK Phage[™] method uses a combination of Immuno-Magnetic Separation (IMS) and bacteriophage (phage) lysis to achieve the required specificity. High assay sensitivity is delivered by the unique AK Bioluminescence assay, which has a limit of detection < 100 CFU.

The method consists of the five stages illustrated in the Alaska Method figure opposite. Samples of a variety of food types were tested by this method (70% by manual, 30% by automated washing) and the results compared to a conventional culture method (CCM). 96 of the samples were tested at an external laboratory (CCFRA²), the rest at Alaska's lab. The results show this method to be as effective as the CCM but able to be completed in a working day compared to 3-5 days for the CCM.

AK BIOLUMINESCENCE

AK is an essential cellular enzyme which catalyses the equilibrium reaction:



Supplied with purified ADP *in vitro*, AK will generate ATP which can then be measured by the bioluminescent reaction of recombinant firefly luciferase (Fig.1).

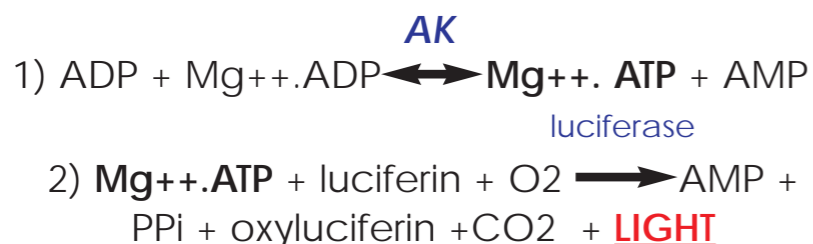
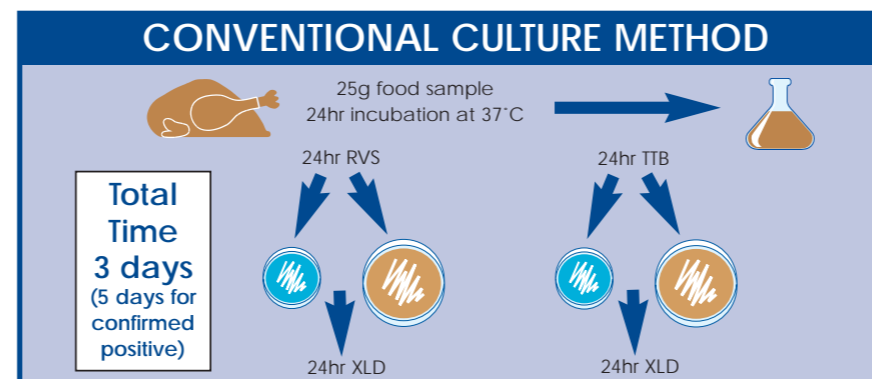
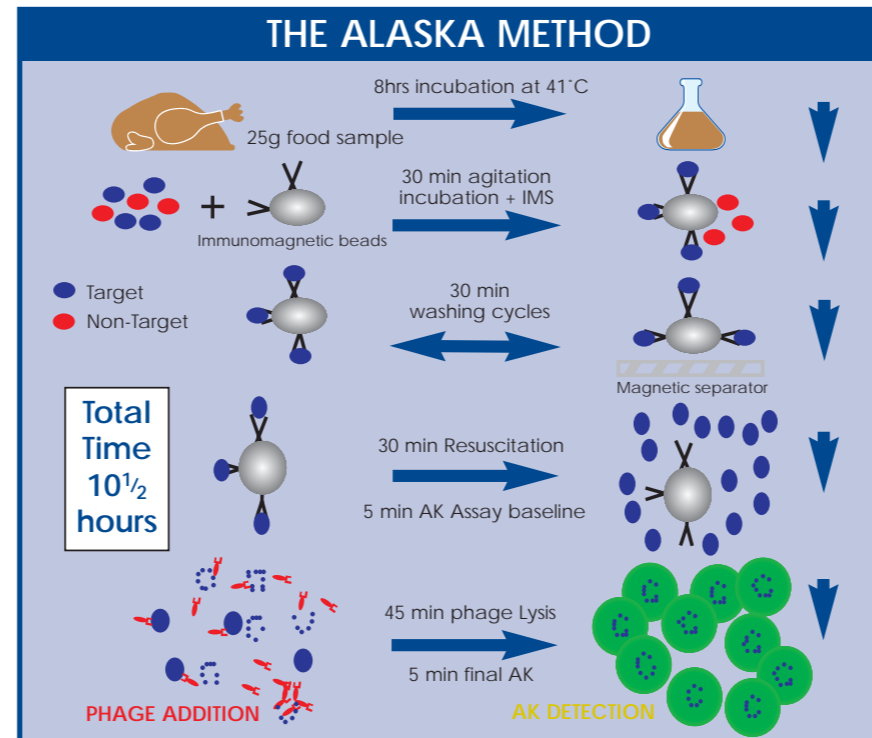


Fig. 1 – AK Bioluminescence



METHOD & MATERIALS

317 paired samples of a variety of raw, processed and Ready-to-eat (RTE) food were analysed by the AK Phage[™] and CCM (Table 1). The TVC background of the foods ranged between <10 CFU/g to 10⁷ CFU/g and the pH between 5.42 ± 0.05 to 7.56 ± 0.05. The food samples were spiked with six different *Salmonellae* at levels between 0.6 and 13 CFU/25g. Some *Salmonellae* were heat stressed by incubating the pure cultures at 51.5°C for 10 minutes; others were cold stressed in the food samples by overnight fridge storage. All samples were confirmed by the CCM.

RESULTS

Within 10½ hours the Alaska AK Phage[™] method gave equivalent results to the CCM in 283 out of 309 samples, indicating a retest in the remaining 8 samples. There were 12 false-positive and 14 false-negative results (Table 1) yielding an overall sensitivity of 94% and a specificity of 86% compared to the CCM. In the more recent samples tested using the automated fastrAK washer (30%), the specificity of the method improved compared with the samples using the manual washing method (70%).

Table 1

Food type	No.	T+	T-	F+	F-	Retest
Bakery	8	6	1	1	0	0
Dairy	52	36	11	0	4	1
Fish	32	27	4	0	0	1
Meat	40	32	4	1	3	0
Misc.	17	12	2	1	1	1
Pasta	35	18	16	0	0	1
Poultry	121	71	32	9	5	4
Veg.	12	7	4	0	1	0
Total	317	209	74	12	14	8

T+ (T): True-positive(-negative). F+ (F): False-positive(-negative), c/w the CCM.



CONCLUSIONS

The report of the CCFRA trial concluded that the Alaska AK Phage[™] method performed well against a conventional culture method for the detection of low numbers of *Salmonella* in a range of foods. The assay yielded positive results within a working day, compared to the conventional method, which required 3 days to yield a confirmed negative result and over 5 days for a confirmed positive result.