IMS/ELISA: A RAPID DETECTION FOR *SALMONELLA*

CUYPERS E.*, TEN BOSCH C.**, VAN DER PLAS J.** and HAAGSMA N.*

* Department of the Science of Food of Animal Origin, Faculty of Veterinary Medicine, Utrecht, The Netherlands. ** Division of Agrotechnology and Microbiology, TNO Nutrition and Food Research, Zeist, The Netherlands.

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Introduction

A modern detection method for *Salmonella* in food samples is the enzyme-linked immunosorbent assay (ELISA). Using a commercial (sandwich) ELISA kit the sample enrichment takes 48 hours (including a pre-, selective and postenrichment), followed by a detection in 2-3 hours. Immunomagnetic separation (IMS) was recently introduced as a versatile tool for isolation and concentration of bacteria (Ø. Olsvik et al, 1994). A combination of IMS and short enrichment was shown to be effective for detection of *Salmonella* by the Polymerase Chain Reaction (PCR) (C. ten Bosch et al., 1993). We have modified the original enrichment (Salmonella-TEK®) into a 20 hours step and combined it with IMS and detection by the commercial Salmonella-TEK® ELISA kit. The whole procedure can be completed within 27 hours (Figure 1). Addition of a filtration step to remove interfering components enhanced the performence of the IMS. For improvement of the sensitivity of this assay immunomagnetic beads were tested as solid phase instead of the microtitre plate used in the first step of the Salmonella-TEK® ELISA.

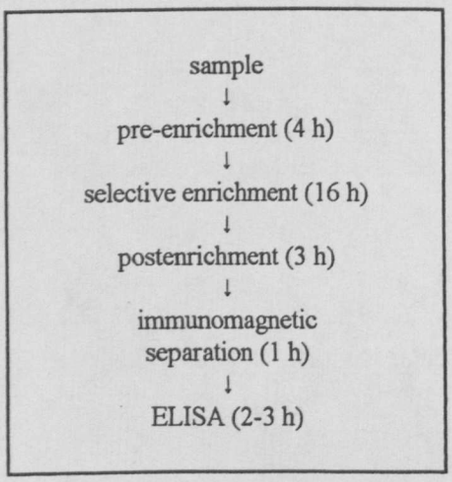

Figure 1. Overview of the procedure for isolation and detection of *Salmonella* in minced bovine meat.

Materials & methods

Minced bovine meat (25 g) was homogenised in buffered peptone water (BPW). After 4 hours pre-enrichment incubation was continued for 16 hours in a selective medium. Positive enrichment cultures were mimiced by addition of different concentrations of *Salmonella livingstone*. Mannose-broth (M-broth, Difco) was supplemented into this selective culture and the incubatin was extended for 3 hours. Prior to immunomagnetic separation (IMS) the culture was filtered to separate bacteria from meat components. IMS was performed using Dynabeads® (M-280, Dynal, Norway) coated with polyclonal antibodies (BacTrace® CSA-1, KPL, USA). 6-7·10^6 beads were added to 1 ml culture and rotated for 30 minutes at 20°C. A magnetic particle collector (MPC®, Dynal) was used to collect the beads and the beads were washed three times in PBS + 0.1% BSA. Finally the beads were resuspended in 100 µl PBS and boiled for 10 minutes. To detect *Salmonella* the commercial Salmonella-TEK® ELISA kit (Organon-Teknika, Belgium) was used.
The use of immunomagnetic beads as solid phase instead of the first step of the Salmonella-TEK® was tested. To detect the bacteria bound to the beads a second enzyme-linked antibody of the Salmonella-TEK® kit or Salmonella BacTrace® was directly applied to the beads after IMS.

Results

postenrichment in M-broth
Antibodies used in the Salmonella-TEK® ELISA are directed against flagelar antigens. To stimulate the flagel production a postenrichment step of 6 hours in M-broth is recommended. Reduction of this incubation time was investigated by adding M-broth into the selective enriched medium. A reconstituted selective enrichment broth was prepared by inoculation of 3·10^5 cfu/ml *S. livingstone* in selective medium with or without M-broth. Samples were tested in the ELISA after 1, 2 and 3 hours incubation at 37°C (Table 1). A positive ELISA was only found after 3 hour incubation in the selective medium with M-broth. No positive results were found when incubated in the selective medium.

incubation time	Ext. (λ = 450 nm)
selective medium	
1 h	-
2 h	-
3 h	-
selective medium with M-broth	
1 h	
2 h	-
3 h	-
	+

Table 1. *S. livingstone* (3·10^5/ml) inoculated in a selective medium and incubated for 1, 2 and 3 hours with or without addition of M-broth. - = negative result; + = positive result

IMS

For improvement of the sensitivity of the ELISA IMS was conducted to concentrate *Salmonella* and to remove meat particles which can interfere with the ELISA. The combination of shortened enrichment, postenrichment in M-broth and IMS was tested for detection with the Salmonella-TEK® ELISA kit. A concentration of 10^6 *Salmonellae*/ml inoculated into the selective medium could be detected in the ELISA without further enrichment or concentration steps. A combination of a 3 hours postenrichment in M-broth added to the selective medium and IMS was necessary to detect 10^5 *Salmonellae*/ml using the ELISA test (Table 2).

25 g minced meat + 4 h pre-enrichment + 16 h selective enrichment	concentration *S. livingstone* added to o/n culture (cfu/ml)			
	$1 \cdot 10^6$	$1 \cdot 10^5$	$1 \cdot 10^4$	blanc
+--	+	-	-	-
+ IMS	+++	-	-	-
+ 3 h M-broth	++	-	-	-
+ 3 h M-broth + IMS	+++	+	-	-

Table 2. Different concentrations of *S. livingstone* added to selective enriched culture and incubated 3 hours with or without additon of M-broth or IMS. Dection was performed by the Salmonella-TEK® ELISA kit. - = negative result; + = positive results.

beads as solid phase

In order to increase the sensitivity of the ELISA even further and to reduce the detection time initial tests were conducted to use immunomagnetic beads as solid phase instead of a microtitre plate. After IMS, the second enzyme-linked antibody was applied to the bead-bacteria complex. Using beads as solid phase the detection level of the ELISA could be decreased 10 times to 10^4 cells/ml. The non-inoculated samples however, gave high background values (Table 3.). A more efficient washing, as with a micrtitre MPC® (Magnetic Particle Collector), might result in a decrease of the background and larger numbers of samples might be processed at the same time.

25 g minced meat + 4 h pre-enrichment + 16 h selective enrichment + 3 h postenrichment in M-broth	concentration of *S. livingstone* added to the selective enriched culture (cfu/ml)			
	$3 \cdot 10^6$	$3 \cdot 10^5$	$3 \cdot 10^4$	blanc
Salmonella-TEK®				
- IMS	+	-	-	-
+ IMS	++++	+++	-	-
Beads as solid phase				
+ IMS, BacTrace® Ab	++++	++++	++++	++
+ IMS, Salmonella-TEK® Ab	+++	++	+++	+

Table 3. Comparison of IMS combined with the Salmonella-TEK®ELISA and the beads as solid phase for *S. livingstone* inoculated in minced meat.
- = negative result; + = positive results.

Conclusions

Modification of the enrichment procedure using a shortened pre-enrichment and addition of M-broth to the selective enrichment reduced the detection time from 48 hours to 27 hours. This combination of short enrichment and immunomagnetic separation resulted in a 10-fold decrease of the detection level for *Salmonella* in minced meat.
The sensitivity of the assay could be improved for another 10-fold when beads were used as solid phase and the enzyme-linked antibody was applied directly to the beads.

References

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