

## Enrichment-ELISA for Detection of *Salmonella typhi* From Food and Water Samples

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**Objective** Development of monoclonal antibody based sandwich enzyme linked immunosorbant assay (sELISA) for rapid detection of *Salmonella enterica* serovar *typhi* (*S. typhi*) from food and water samples and optimization of enrichment procedures for use with the developed sELISA to increase the detection sensitivity of the assay. **Methods** Spleen cells from BALB/c mice immunized with flagellin (H=d) antigen of *S. typhi* were fused with Sp2/0 myeloma cells. The hybridoma cell line specific to H=d antigen was established, characterized and ascites raised against one of these clones. The hyperimmune serum to flagellin antigen was raised in New Zealand White rabbits. An sELISA was developed using polyclonal antibody as capture and monoclonal antibody as detection antibody. To design the efficient culture strategies for use with the sELISA, different pre-enrichment and enrichment broths were evaluated. The media included buffered peptone water (BPW) and brain heart infusion broth for pre-enrichment and selenite F broth and Rappaport-Vassiliadis broth as enrichment broths. The developed sELISA with preceding enrichment step in BPW (Enrichment-ELISA) was evaluated in various food samples artificially inoculated with *S. typhi* bacteria. Various food (30) and water (35) samples collected from field were also tested by Enrichment-ELISA and culture method. **Results** Out of four specific clones to H=d antigen, one clone (# 2/56, IgG2a isotype) was used in sELISA. The sELISA had the detection limit of  $10^4$ - $10^5$  cfu of *S. typhi*. Of the various broths used with sELISA, BPW was found to yield maximum ELISA values. Enrichment-ELISA, when tested in artificially inoculated food samples, generally, could detect  $10^2$  *S. typhi* cfu/mL within 10 h from various food rinses (meat, vegetable) and milk samples. After overnight enrichment in BPW, as less as 2 bacteria per 10 mL of milk, meat rinse, and chicken rinse could be detected. Only one of the field samples (water) gave false positive result by Enrichment-ELISA. **Conclusion** In comparison to culture, the Enrichment-ELISA is a rapid, sensitive, and specific method for detection of *S. typhi* from food or water samples. This method may be used as rapid screening procedure for environmental monitoring during outbreak situation.

**Key words:** Enrichment-ELISA; Food; Monoclonal antibody; *Salmonella*; Sandwich ELISA; Water

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