Elimination of Cross Reactions During Detection of Salmonella spp., with Commercial ELISA Kits

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Abstract: In this paper we first speculated and later on have proved that the increased number of Salmonella positive results, using the Salmonella Tek-Elisa method as compared to the standard technique, was due to false positive results of the former method. We found out that the higher sensitivity of the former method was due to P. stuartii E. cloacae and E. coli bacteria (CRBs), which cross-reacted with the monoclonal antibodies of the method, giving rise to a higher number of Salmonella false positive results. The purpose of the present study was to identify the source of the higher sensitivity of the Elisa Tek technique and propose corrective steps.

Keywords: Immunoassays, Elisa, Salmonella-Tek

Introduction: The standard microbiological method for the isolation of Salmonella spp. is extensively used around the world to study foods of animal origin, faeces, and/or animal feeds. Rapid methods are also being used as well. Among the rapid methods that are used for the identification of Salmonella spp. are the Enzyme linked immunoabsorbent assays (Elisa), genetic, immunomagnetic separation and, electrical conductance methods (Fierens, and Huyghebaert, 1996, Kofitsyo, and Crona 1997). Some of the minor disadvantages include the time required to complete the procedure as well as the sensitivity of the method. The Elisa method is based on the antigen-antibody reaction. So if a sample has any Salmonella spp. antigens, they will attach to the specific (monoclonal or polyclonal) antibodies which are designed for Salmonella spp. antigens and coated to polystyren microcells. By using a suitable algorithm made by designer of the method a threshold value is being calculated. Any sample over the threshold is considered positive for Salmonella spp. (Mattingly, and Gehle, 1984). The most common problem arising from the use of such rapid techniques is the cross reactivity of the Salmonella spp. antibodies with the antigens of other enterobacteriaceae bacteria. The Salmonella-Tek Elisa method seemed to be the most susceptible to cross reactivity problems even though the method uses monoclonal antibodies specifically designed for Salmonella spp. (Beckers, et al, 1998). In a previous study (Limpitakis, et al, 1999) we tested 1878 environmental and product samples collected in 2 slaughterhouses by the standard microbiological and the commercial
Salmonella Tek- Elisa. In 178 (9.5%) of the samples we isolated Salmonella spp. When 1657 samples evaluated by both the microbiological and the Salmonella Tek- Elisa™ methods then 154 (9.3%) and 420 (25.3%) were found positive for Salmonella spp. respectively. The above findings indicate the presence of a problem with respect to the sensitivity and specificity among the methods and especially the Elisa Tek, which had the highest sensitivity.

Materials and Methods:


Results: A big difference in the number of Salmonella spp. positive samples from the environment and products of 2 slaughterhouses was found between the standard microbiological and the Salmonella Tek- Elisa™ methods. Using microbiological isolation methods we investigated the M-Broths and especially samples where the Elisa gave a positive reaction for Salmonella and the standard microbiological method did not isolate any Salmonella from the original samples. From the M-Broths we took aliquots and spread them on Nutrient agar. Using the API20E diagnostic kit from isolated colonies 3 bacterial species were identified as being the P. stuartii, E. cloacae, and E. coli. Those bacteria were able to overcome the selective environment of RV, SC and M-Broths. The CRBs were next subjected to an antibiotic susceptibility test. The antibiotics Gentamycin, Sulfmethoxazole-Trimethoprine, and Nalidixic acid have been identified as the ones to which the Salmonella spp. bacteria were resistant and the CRBs were sensitive to. It is well known that at least 10^6 Salmonella cells per ml of M-Broth are required (threshold) for its detection by the Elisa techniques (Blackburn, et al, 1994). By keeping the individual CRBs lower than this threshold level in the M-Broth we minimized the possibility of cross reactions and thus false positive results. The use of antimicrobials as early as possible in the methodology is critical in order to block potential growth of CRBs. It has been shown that the probability of a bacterium to overcome the inhibitory environment of the selective enrichment media is dependent on its initial concentration of cells (Fernandez–Garayzabal, and Genigeorgis, 1990). Thus the higher the initial numbers the greater the probability of growth initiation and the more the difficulty for their inhibition by antimicrobials. We tested the probability of one of their cells to initiate growth in selective enrichment media including RV, SC, and Tetrathianate Bile Brilliant Green/TBG (Eckner, et al, 1994). The experiments revealed that the TBG, was toxic enough to inhibit the growth of all three CRBs if used alone. However the Salmonella- Tek Elisa protocol does not allow the use of a single enrichment broth but the combination of two, namely RV and SC. In probability studies we found that if the CRBs managed to grow in the BPW to ≥10^2/ml then the RV and SC could not block their growth and allow them eventually to reach levels grater than
the threshold (≥10⁶/ml) in the M-Broth. Therefore in order to control the population of *P. stuartii* in the BPW without affecting *Salmonella* spp. growth Nalidixic acid at the concentration of 15mg/L BPW had to be used. To control the growth of *E.coli* & *E.cloacae* 5mg/L BPW of Gentamycin should be used in combination with Nalidixic acid. The combination of the two antibiotics was able to reduce the initial inoculum of *P. stuartii* in the BPW by 5 logs and *E.coli* & *E.cloacae* by 7 logs.

**Discussion**: The proposed changes in the original methodology in order to eliminate cross reactions and false negative results has as follows: a) Pre-enrichment in BPW with Gentamycin and Nalidixic acid, b) Selective enrichment in TBG and RV, c) Inoculation of M-Broth with a mixture of 1 ml TBG+ 1ml RV. The proposed methodology not only eliminates the problem of cross-reactions in Elisa but it can be used also in the standard microbiological isolation method for *Salmonella* to enhance its sensitivity, especially in samples with extensive microbial competition like environmental and feacal samples.

**References**
(ISO 6579/1993, BS 5763 Part 4. Detection of *Salmonella*).