Scientific Interpretive Summary: Revalidation of screen test kits for *Escherichia coli* O157:H7 - Follow-up study

In response to recent initiatives by the United States to further control *E. coli* O157:H7 in the US beef supply, NZFSA and the New Zealand industry agreed with FSIS to implement changes to its microbiological monitoring programme to facilitate continued market access to the US. Changes included an increase in analytical sample size, a change of enrichment broth, a reduction in volume of enrichment broth, an increase in incubation temperature to 42±1°C for all screen methods, and a reduction in incubation time from 18-24h to 15-22h.

A previous NZFSA/AsureQuality study was carried out to verify that the performance of three of the *E. coli*O157 screen kits (Reveal 20h, VIP and Tecra VIA) then approved by NZFSA and the FSIS specified screen kit (BAX MP) was not adversely affected by the modified sample and enrichment procedures. The study revealed that the lateral flow screen kits (Reveal 20h and VIP) showed weak, difficult to read, reactions for some strains at 15h; the VIP kit reaction remaining weak after 22h incubation.
A follow-up study was commissioned to investigate these observations using the three strains of *E. coli* O157:H7 that expressed weak reactions in the original work.

The findings of the original study could not be reproduced in that all samples inoculated with *E. coli* O157:H7 were positive in the screen methods evaluated. However, false negative results were encountered in one of the replicates in two veal samples, whereby all screen kits including Tecra, which performed well in the original work, failed to detect *E. coli* O157:H7. The cultural plating method confirmed the presence of *E. coli* O157:H7 at levels of ~1.0X10^5 cfu/ml in the sample enrichment broth, but high levels of background flora were also noted. It was concluded that the original veal matrix contained high levels of background microflora which likely “out competed” the target organisms during enrichment and led to a masking effect in the subsequent screen tests.
Additional work was performed to compare the selective performance of the old enrichment media (mEC) against the new media (TSB+NCA) when increased levels of background flora are present in meat samples. All samples with high background flora that were spiked with *E. coli* O157 tested negative by all screen kits at every incubation time point. Both media failed to suppress the competitive flora present in the beef samples with high background flora. In contrast, every sample with low background flora that was spiked with *E. coli* O157 returned a positive result for all screen kits at the earliest time point evaluated in this study, 15 hours. The results demonstrate equivalence between the two enrichment media with respect to selectivity but limitations of each formulation to suppress gram negative competitive flora.

The limit for detection of *E. coli* O157using these kits under these conditions appears to be in the order of 1X10^5- 5X10^5 cells, which is not always achieved following enrichment from

the veal samples studied, particularly those with high background bacterial counts. Deficiencies of enrichment protocols for *E. coli* O157:H7 have been reported previously and are currently only overcome using more sensitive detection systems (e.g. IMS-PCR). There is evidence from the New Zealand National Microbiological Database monitoring programme that the level of background bacterial flora on veal from very young calves is higher than on beef. It is possible differences in levels and/or composition of background flora on beef and veal occur between New Zealand and overseas countries, although there is currently no evidence in support of this contention. However, this study highlights the potential difficulties that may result when applying *E. coli* O157:H7 screen kits validated in other countries, to samples from New Zealand beef and veal from very young calves.

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