Final Report

AMPC
AUSTRALIAN MEAT PROCESSOR CORPORATION

dd-Check STEC Solution evaluation for use in export meat testing

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Contents

1.0 Abstract

Why was the project undertaken:

- Testing of beef products for the presence of Top7 Shiga toxin-producing *E. coli* (Top7 STEC) typically relies on detecting genes that encode Shiga toxin (*stx*), intimin (*eae*), and specific O-antigens (O26, O45, O103, O111, O121, O145, and O157). These three genes can be carried by bacteria in different combinations, which together may trigger a potential positive (PP – defined as a broth that is positive for *stx*, *eae* and O-antigen genes) broth, whereas the individual bacteria would not. There is a substantial difference in the rate of potential positives generated by screening and the number of confirmed positives. Bio-Rad's dd-Check STEC solution is the first system to determine the co-location of *stx* and *eae* which could not only decrease the number of potential positives (PPs) or samples requiring culture confirmation but decrease the turnaround time of STEC testing. The purpose of the project was to evaluate the performance of Bio-Rad dd-Check STEC solution for screening and confirmation of 100 Australian manufacturing beef enrichment broths.

How was the project performed:

- One hundred manufacturing beef enrichment broths identified as PP for a Top7 STEC serogroup were included in the study. Nineteen of the 100 PP manufacturing beef broths were confirmed positive for O157 (12), O26 (3) or O111 (4) using a DAFF (Department of Agriculture, Fisheries and Forestry) approved culture confirmation method. Each broth was subsequently screened using the Bio-Rad IQ-Check detection system for *stx*, *eae* and Top7 serogroups and tested by the Bio-Rad dd-Check for the co-localisation of *stx* and *eae*.

Key Results:

- IQ-Check performance: There was good correlation (98%) between samples determined PP by PCR using FSIS Guidebook MLG5C Appendix 4.0 (MLG5C) primers and probes and IQ-Check.
- dd-Check performance as a screening tool: There was a 68% reduction in the PP rate compared to traditional STEC screening systems. However, two culture-confirmed samples may have been missed because the O serogroup likely fell below the dynamic range of dd-Check, a consequence of the necessary dilution during sample preparation due to high *stx* levels.
- dd-Check performance as a confirmatory tool: dd-Check confirmed 24 broths as containing a Top7 STEC. An additional eight were presumptive positive and would require culture confirmation. Compared to traditional methods this is a 26% increase in confirmed samples. This increase is likely due to the presence of non-Top7 STEC carrying *stx* and *eae*. Expansion of the dd-Check solution to enable co-location of *stx*, *eae*, and O serogroups would likely reduce this number.

Benefits to industry of the results:

- Using dd-Check as a screening tool could lower potential positive (PP) rates, helping to reduce labour demands, minimise unnecessary product holds and reduce the number of PP requiring culture confirmation.
- Using dd-Check as a confirmatory tool allows for faster turnaround with results typically available within a day of sample collection.
- A cost-benefit analysis is needed to assess its overall value to the industry.

- Expanding dd-Check to include the co-location of *stx*, *eae* and O-serogroups could further reduce the PP rate.

2.0 Executive summary

Purpose of the study

This project evaluated the performance of Bio-Rad's IQ-Check and dd-Check STEC Solution for detecting and confirming Top7 Shiga toxin-producing *Escherichia coli* (STEC) in Australian beef manufacturing broths. Traditional STEC testing occurs in two stages, firstly the enrichment is screened for the presence of three gene markers: *stx*, *eae*, and a Top7 serogroup, typically using BAX, GDS or Gene-up. If all three genes are detected in a broth, the broth is deemed potentially positive (PP) for a Top7 STEC. This screening method cannot determine if all three targets are present in a single organism, therefore a culture confirmation step is required. There is a substantial difference in the rate of potential positives (PP) generated by screening and the number confirmed by culture confirmation. This two-stage screening and culture confirmation can be lengthy so there is a desire to have a rapid process, that also reduces the number of potential positive samples that require confirmatory testing. The dd-Check STEC solution offers a potential solution to this challenge, that involves a novel approach to improve confidence in the potential positive status of a broth by demonstrating the co-location of *stx* and *eae* within a single bacterial cell. The aim of this project is to evaluate the utility of the dd-Check STEC solution as an STEC screening and confirmatory tool.

Objectives

- Evaluate the performance of the Bio-Rad dd-Check STEC Solution on manufacturing beef enrichment broths that have previously been identified as potentially positive, using either the Bax® (BAX), GDS® (GDS), or Gene-Up® (Gene-Up) test systems.

Methodology

- Beef enrichment broths were collected from laboratories using DAFF-approved methods for the detection of Top7 STEC (BAX, GDS, and Gene-Up).
- Samples were screened for the virulence genes *stx* and *eae*, as well as the Big6 STEC serogroups (O26, O45, O103, O111, O121, O145) using FSIS MLG5C primers, and for O157 using primers described by Perelle et al (Perelle et al., 2004). Samples were included in the study if they were classified as potentially positive (PP), resulting in a final set of 100 samples.
- All 100 PP samples underwent VirX screening for *stx* and *eae* virulence genes and SerO screening for Top7 serogroups.
- The dd-Check STEC solution was applied to all PP samples to confirm the co-location of *stx* and *eae* genes within a single bacterium.
- Quantitative PCR (qPCR) was performed to determine the concentration of *eae* and O serogroups present in samples.

Results/key findings

- IQ-Check performance demonstrated 98% correlation with FSIS MLG5C PCR-based detection using FSIS MLG5C primers and probes.
- dd-Check performance as a screening tool achieved a 68% reduction in PPs compared to conventional STEC screening systems. However, two culture-confirmed samples were not

identified as PPs likely due to dilutions carried out during dd-Check sample preparation required as a result of high *stx* concentrations in the sample., The dilution may subsequently have caused the O-serogroup concentration to fall below dd-Check's detection range.
- dd-Check as a confirmatory tool identified nine additional positives, likely due to the presence of non-Top7 STEC strains. The expansion of the dd-Check solution to enable the co-localisation of O serogroups to also be demonstrated would likely reduce this number. Additionally, it did not eliminate the need for culture confirmation entirely as the dd-Check flagged eight additional presumptive positives requiring culture confirmation.

Benefits to industry

- As a screening tool it could significantly reduce the PP rate, reducing the number of broths that would need to proceed for culture confirmation.
- dd-Check as a confirmatory tool could enable next-day confirmation for dd-Check non-presumptive positives, reduce delays and minimise unnecessary product holds.

Future research/extension/adoption and recommendations

- Conduct a cost-benefit analysis to determine whether dd-Check is a financially viable option for industry to use as a screening or confirmation method.
- Expanding dd-Check to include co-location of *stx*, *eae*, and O-serogroups may help address dynamic range limitations and improve confidence in confirmation reliability. Additionally, incorporating alternative markers could further reduce the rate of potential positives.
- For industries considering adoption, it may be beneficial to incorporate dd-Check as a complementary screening tool alongside their existing screening method. This approach could provide a comprehensive understanding of the dd-Check solution's advantages, including cost-effectiveness, turnaround time, and overall performance.

3.0 Introduction

The United States Department of Agriculture Food Safety and Inspection Service (USDA - FSIS) considers O157 Shiga toxin-producing *E. coli* (STEC), and six additional non-O157 serotypes (O26, O45, O103, O111, O121 and O145) as adulterants of raw non-intact beef products. The US has a 'zero tolerance' policy for the presence of these Top7 STEC in raw non-intact beef, and any lots testing positive at port-of-entry will not be permitted entry to the US market (Sumner et al., 2011). Australia is a leading supplier of raw manufacturing beef to the United States, where it is primarily used for grinding into hamburgers. To comply with US regulations Australian export-registered meat abattoirs must verify that each lot of manufacturing beef tests negative for Top7 STEC.

In Australia, STEC testing must be performed using a method approved by the Department of Agriculture, Fisheries and Forestry (DAFF)(Department of Agriculture, 2025b) and conducted at a DAFF-approved laboratory (Department of Agriculture, 2025a). Current testing involves screening enriched meat broths for two virulence genes, *stx* and *eae*, as well as for the presence of one of the Top7 STEC serogroups. If all three targets are detected, the sample is classified as potentially positive (PP). However, the presence of these genetic markers during screening does not necessarily confirm the presence of STEC, as the genes may be carried by a combination of non-STEC organisms (Capobianco et al., 2020). In Australia, Top7 STEC screening is typically carried out using BAX, GDS or Gene-Up real-time PCR systems. Real-time PCR cannot determine if *stx* and *eae* are co-located in the same organism, therefore a culture confirmation step is required to verify

the sample contains a Top7 STEC. The rate of PP samples that confirm is low due to the presence of these markers in nonTop7 *E. coli* and in Top7 STEC carrying just *stx* or *eae* (Capobianco et al., 2020).

New approaches to STEC detection have been developed by Bio-Rad Laboratories. The Bio-Rad dd-Check Solution kit uses Bio-Rad's digital droplet technology to demonstrate the co-location of *stx* and *eae* in the same bacterium allowing a reduction in the number of potential positives requiring culture confirmation.

The Bio-Rad digital droplet system uses oil emulsion technology to encapsulate individual cells into 20,000 nano-sized droplets. The droplets act as partitions, and PCR amplification is carried out within each droplet within the same sample. Following PCR, the droplets are streamed single file into the QX200 Droplet reader which counts the number of positive and negative droplets by measuring fluorescence. The software determines the number of droplets that are positive and negative for each fluorophore FAM (*stx*) and VIC (*eae*) in each sample. The QxIDE software uses Poisson distribution to calculate the gene copy number based on the fraction of positive and negative droplets. The double-positive droplets are used by the software to calculate the linkage percentage for the sample (Bio-Rad Laboratories). The linkage analysis shows if *stx* and *eae* are co-localised in the single bacterium allowing culture independent differentiation from unlinked virulence markers.

This approach has the potential to enhance the specificity of the detection system and subsequently reduce the frequency of potential positive broths that are not confirmed by culture tests. This project aims to assess the performance and effectiveness of the Bio-Rad System and provide data that can support decisions by the Department of Agriculture, Fisheries and Forestry to approve the test system for use in Australia.

4.0 Project objectives

Evaluate the performance of the Bio-Rad dd-Check STEC Solution on manufacturing beef enrichment broths that have previously been identified as potentially positive, using the Bax (Hygiena), GDS (Bio-Control), or Gene-up (BioMérieux) test systems.

5.0 Methodology

5.1 Initial Sample Collection and Screening

Samples were collected between April and August 2025. Enrichment and original screening were conducted either at on site plant laboratories (abattoir) or at off premise laboratories. All samples included in this study were initially screened and determined to be PP for Top7 STEC (O26, O45, O103, O111, O121, O145 or O157) using a DAFF approved method BAX (Department of Agriculture, 2025d), GDS (Department of Agriculture, 2025c) or Gene-Up (Department of Agriculture, 2025e). Briefly, 375 g of manufacturing beef was used for, Bax, GDS and Gene-Up. BAX samples were enriched in 1.5 L of pre-warmed (45–46 °C) BAX System MP enrichment broth and incubated at 39–42 °C for 12–24 hours, GDS samples were enriched in 1.5 L of pre-warmed (42 °C) mEHEC medium and incubated at 42 °C for a minimum of 10 hours and Gene-Up samples were enriched in 1,125 mL of pre-warmed (41.5 ± 1 °C) BPW and incubated at 41.5 ± 1 °C for 10–24 hours.

De-identified aliquots (~25 mL) of manufacturing beef enrichment broths were provided to CSIRO on a weekly basis for the purposes of assessing the Bio-Rad dd-Check STEC solution method. Details of which samples had confirmed (i.e. an STEC had been isolated) and the serogroup to which the isolate belonged were provided to CSIRO. On arrival, boiled cell lysates (BCL's) were prepared for all manufacturing enrichment broths. A 1 mL aliquot of broth was centrifuged for 3 min at 14,000rpm, supernatant discarded, and the pellet resuspended in 500 µL of sterile water, boiled for 10 min and the centrifugation step repeated (Barlow et al., 2004). All DNA preparations were screened for *stx*, *eae* and the Big 6 serogroups via real time PCR as per the FSIS Guidebook MLG5C Appendix 4.0 (MLG5C) (United States Department of Agriculture Food Safety and Inspection Service, 2019) and for O157 as per Perelle (Perelle et al., 2004). Samples were included in the final set of 100 once they tested positive to *stx*, *eae* and any Top7 serogroup.

5.2 Bio-Rad Screening using VirX and SerO

All potential positive samples were tested for STEC virulence genes stx_1/stx_2 and *eae* using the IQ-Check STEC VirX Kit Real-Time PCR as per the user guide10000131516.pdf (bio-rad.com). In brief, to prepare the DNA extract, 100 µL of homogenous lysis reagent (reagents A + F) was aliquoted into each well of a deep well plate and 100 µL of enrichment broth was added. The sample was incubated at 95–100°C for 15–20 minutes at 1,300rpm in the thermoshaker (Easy I Protocol). The deep well plates were cooled prior to PCR analysis. The PCR mix was prepared with 5 µL of probe (reagent B) and 15 µL of amplification mix per sample. The IQ-Check STEC VirX Kit PCR mix calculation guide was used to calculate the volume of mix required allowing for a positive and negative control per run. Aliquots of 20 µL of PCR mix plus 5 µL of sample was added to the appropriate wells of the PCR strips, along with positive (reagent E) and negative controls (reagent D). The PCR was sealed and run on the CFX Optus deep well real-time thermal cycler and the results analysed on the CFX Manager IDE Software. For the PCR run to be valid controls had to yield a Cq 26 ≤ Cq ≤ 3 or the run was repeated.

The IQ-Check STEC SerO II Kit is a real time PCR test for the detection of the Top7. The DNA extract prepared for the IQ-Check STEC VirX PCR was used. Three PCR mixes SerO1, SerO2 and SerO3 were prepared for each probe B1 (O157:H7 and O111), B2 (O26, O103 and O145) and B3 (O45 and O121) using the IQ-Check STEC SerO II Kit PCR mix calculation guide. Aliquots of 20 µL of PCR mix plus 5 µL of sample was added to the appropriate wells of the PCR strips, along with positive and negative controls. The PCR was run on the CFX Optus deep well real-time thermal cycler and the results analysed on the CFX Manager IDE Software. For the PCR run to be valid controls had to yield a Cq 26 ≤ Cq ≤ 3 or the run was repeated.

5.3 dd-Check Methodology

The 100 PP samples were tested with the dd-Check STEC solution kit which detects the co-location of virulence genes *stx* and *eae* in a single bacterium. A 1 mL aliquot of the beef enrichment broth was filtered with a 10 µL filter into a new tube. The filtered sample was centrifuged at 6,000g for 5 minutes. The supernatant was removed, and the pellet was resuspended in 200 µL of BPW. Digital droplet PCR (ddPCR) samples were prepared by adding 18 µL of the Bio-Rad PCR Mix (reagent ddA + ddP) and 6 µL of the sample into a well. Sterile BPW was used as a negative control and reagent ddC as a positive control in each plate setup. The ddPCR samples were gently mixed 5 times with a pipette and 20 µL of each were transferred into individual wells of the droplet generator cartridge. 70 µL of droplet generation (DG) oil was added to each well and the drops generated using

the QX200 Droplet generator. To prepare the samples for PCR amplification, 40 µL of the droplets were transferred from the cartridge into a PCR plate with an 8-channel pipette. The PCR plate was sealed with the PX1 PCR plate sealer, and the protocol run on the PTC Tempo deep well thermal cycler. The plate was loaded into the QX200 Droplet Reader, and the linkage analysed with Qx IDE Manager Software determining if both virulence markers coexist in a single drop.

5.4 Quantitative PCR

The concentration of *eae* and O-antigen genes was determined using the primer and probes used in FSIS MLG5C and Perelle et al. (see above) and compared to a standard curve. Two standard curves were prepared, one for Top7 serogroups and one for *eae*. Reference sequences for Top7 serogroups and *eae* were sourced from the NCBI database. Primer regions specified in the FSIS MLG5C and Perelle et al. (Perelle et al., 2004) were mapped to corresponding sequences. For each target, a gene fragment was designed by extracting 20 base pairs upstream and downstream of the primer binding sites used in FSIS MLG5C and O157 detection protocols. Each gene fragment was combined to form a single construct. A spacer consisting of five thymine bases (5T) was inserted between each gene fragment to identify the start and end of each gene fragment. The final sequence was synthesised as a gBlock by Integrated DNA Technologies (IDT). The gBlock was resuspended according to IDT's standard protocol. The resuspended gBlock was serially diluted in nuclease-free water to generate a standard curve ranging from 10^8 to 10^0 copies/µL. Standard curves were generated using duplicate CT measurements for all dilutions.

6.0 Results and Discussion

The objective of this study was to evaluate the effectiveness of IQ-Check for initial screening and dd-Check as a screening and confirmatory tool for Shiga toxin-producing *Escherichia coli* (STEC) in Australian beef manufacturing broths. There are several options available for those planning to use the Bio-Rad STEC solution in Australia. At the entry level, the IQ-Check Kit (VirX and SerO) serves as a real-time PCR screening tool, equivalent to classical methods like Bax. It screens for the presence of *stx*, *eae*, and the Top7 O serogroups, making it suitable for initial detection of potentially positive samples. Alternatively, Bio-Rad also offers the dd-Check STEC Kit, which uses digital droplet PCR (ddPCR) technology as an innovative screening tool. This kit can determine the co-location of stx and eae within the same bacterium, helping to reduce the potential positive (PP) rate. At the other end of the spectrum, some users may choose to use dd-Check directly as a confirmation tool, although they would still require serogroup information from the SerO kit. The study initially uses VirX as SerO as a classical STEC screening tool to confirm the status of potential positive manufacturing beef broths. It then assesses the utility of dd-Check STEC as an addition to the screening process or for use as a confirmation tool.

6.1 Initial Sample Collection and Screening

Of the 100 PP samples included in the study, 21 were tested at on-plant laboratories and 79 at off-premises laboratories. Of the off-premise laboratory samples, 45 PPs were generated using BAX, four by GDS and 30 using the Gene-Up System. An equivalent breakdown of test system usage was not available for samples screened at beef processing plants. Of the 100 PP samples, 19 were culture confirmed for O26 (3), O111 (4) and O157 (12) STEC using DAFF approved methods (MLG5C.03).

6.2 Bio-Rad Screening using VirX and SerO

Of the 100 samples deemed to be PP by both a DAFF approved method and by MLG5C primers and probes, 98% were also deemed PP by IQ-Check (VirX and SerO results) showing good correlation. Of the two samples that were not PP by IQ-Check, one sample was negative for *eae* but contained *eae* by dd-Check, which is likely a result of variability in PCR. The second sample had a low qPCR concentration (2.73 $\log_{10}$ copies/mL) of O45 serogroup gene and was likely below the limit of detection for IQ-Check. There was also good correlation between the serogroup detected by a DAFF approved methods and SerO with 91% of samples sharing at least one serogroup (see appendix 1).

All 100 PP broths were tested using the Bio-Rad dd-Check STEC Solution which has AOAC international approval (012401) for detecting and confirming virulence genes from STEC by co-localisation of the *stx* and *eae* genes. The dd-Check software gives four possible results: 1) a negative result which indicates one or both virulence genes were missing, 2) a negative unlinked result which indicates *stx* and *eae* are detected but are likely from different bacteria, 3) a positive linked result indicates *stx* and *eae* are colocalised, representing a confirmed STEC and 4.) a presumptive positive sample indicates further testing is required to determine if *stx* and *eae* are collocated in the same bacteria within the sample. A key strength of the dd-Check STEC solution is its ability to deliver next-day confirmation.

6.3 Assessment of dd-Check solution as a Top7 STEC screening tool

In this study, 32 samples were screen PP (Positive: linked targets or Presumptive Positive) by dd-Check. This represents a 68% reduction in the number of PP against classical detection systems and number of samples that would ordinarily go for culture confirmation (Figure 1). However, two culture confirmed samples did not give linked results (#24 and #54). On both occasions dd-Check reported high concentrations of *stx* and required dilutions to be prepared and tested. Sample #24 was diluted 1/20 to bring the *stx* concentration to a detectable range for dd-Check. Quantitative PCR (qPCR) can be useful for understanding the relative performance of STEC test systems. Diluting the sample would have reduced the concentration of O157 gene copies to 1.85 $\log_{10}$ gene copies/mL and perhaps reduced the concentration to below the detectable limit of the PCR. Similarly, Sample #54 was diluted 1/50 to bring *stx* into a detectable range for dd-Check PCR. Diluting the sample would have reduced the concentration of O111 gene copies to 3.09 $\log_{10}$ gene copies/mL and perhaps reduced the concentration to below the detectable limit of the PCR.

These findings imply that while *stx* was elevated, the co-located presence of O-serotype, *stx* and *eae* may be limited, and the *stx* signal could be attributed to other bacterial sources. It should also be noted that for all samples, the AOAC 012401 method was not followed which utilises Bio-Rad BPW and a STEC supplement. The broths supplied were grown in propriety media stated in each screen method.

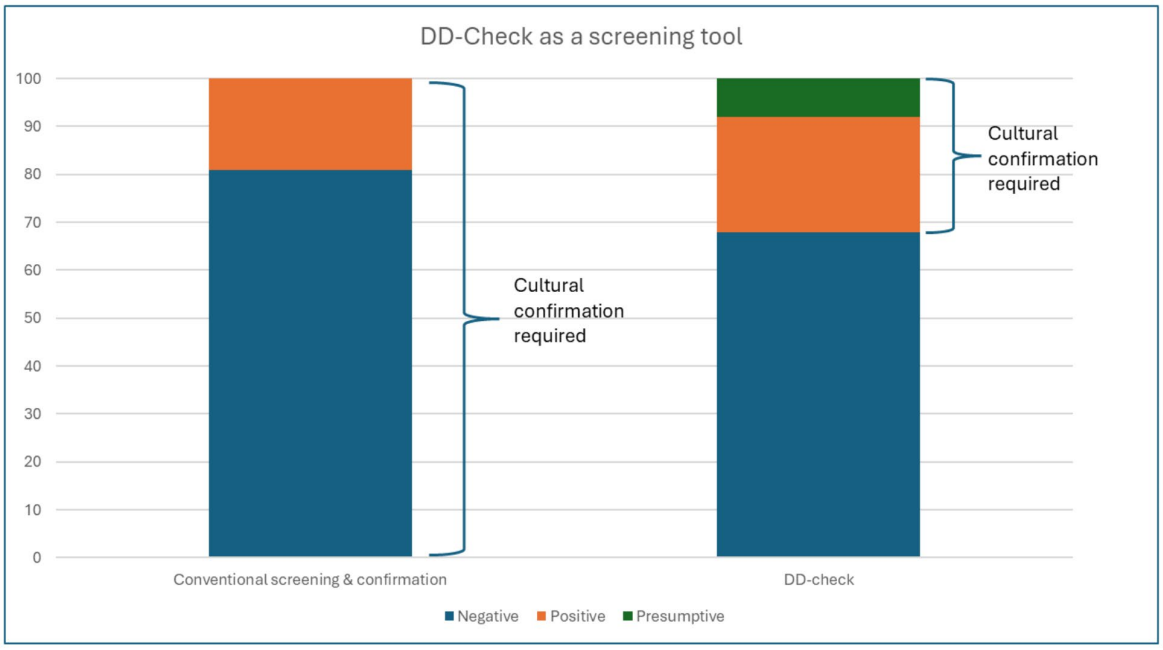

Figure 1. Bar Graph of dd-Check Results compared with traditional screening tools. Presumptive positive = undetermined samples that require additional culture confirmation.

6.4 Assessment of dd-Check solution as a Top7 confirmation tool

All samples were tested by dd-Check for the co-localisation of *stx* and *eae*. Samples giving a positive linked target result are considered confirmed by dd-Check. Presumptive samples need to go for culture confirmation as their confirmation status cannot be determined by dd-Check. To evaluate the dd-Check as a confirmation tool dd-Check positive linked results were compared with culture confirmation results supplied by test laboratories (as shown in Table 1).

Table 1. dd-Check STEC Results

dd-Check STEC Result	# of samples	Confirmed by cultural method
Negative	13	0
Negative: unlinked targets	55	2
Positive: linked targets[1]	24	15
Presumptive positive[2]	8	2
Total #	100	19

[1] Considered confirmed and do not require culture confirmation

[2] Require culture confirmation

The results show eight samples gave a presumptive positive result and would need to proceed to cultural confirmation. Of the remaining 92 samples, 24 gave linked result which represents a 74% reduction in samples that would ordinarily go for culture confirmation. However, two confirmed samples did not give linked results (#24 and #54). As discussed above in the assessment of the

system as a screening tool on both occasions dd-Check reported high concentrations of *stx* and required dilutions to be prepared and tested. This may have reduced the concentration of colocalised *stx* and *eae* associated with a Top7 serogroup organism to below detectable limits.

Of the 24 linked target samples, 15 had previously been confirmed by culture confirmation which means that an additional nine samples were confirmed by dd-Check compared with culture confirmation. As dd-Check only confirms linkage of *stx* and *eae* it is possible to hypothesise that these samples contained non-Top7 STEC. The expansion of the dd-Check STEC Solution to include serogroup co-location with *stx* and *eae* would likely reduce this number.

6.5 Quantitative PCR

All PCR-based test systems will have a limit of detection below which a negative result will be obtained. In general, PCR has a theoretical limit of detection of ~4.00 $\log_{10}$ copies/mL and consequently enrichment broths containing target genes at or below this concentration are a challenge for test systems. Test systems may vary in their PCR efficiency and specificity which may affect the ability to identify some gene targets which may ultimately influence the outcome of testing. The mean concentration of *eae* and O-antigen genes for all samples by in-house PCR PPs are shown in Table 2. Along with the minimum and maximum result for all samples. Individual data for all samples is available as supplementary material on request.

Table 2. Mean, minimum and maximum ($\log_{10}$ gene copies/ml) concentration of *eae* and O-antigen gene for all samples

Gene target	*eae*	O26	O45	O103	O111	O121	O145	O157
Concentration	4.5	4.1	3.9	4.6	3.6	4.2	5.4	4.4
Minimum	2.2	2.5	2.3	2.6	2.6	2.9	5.4	3.0
Maximum	7.8	6.4	6.2	7.2	4.8	6.9	5.4	6.4

7.0 Conclusions

- There was good correlation (98%) between samples determined PP by PCR using MLG5C and IQ-Check.
- The use of dd-Check as a screening tool resulted in 68% reduction of PPs in comparison to traditional STEC screening systems. However, two samples were not deemed to be PP, likely due to the O serogroup falling below the dynamic range of dd-Check.
- The use of dd-Check as a confirmation tool may result in additional positives being called that are likely due to the presence of non-Top7 STEC. Expansion of the dd-Check solution to enable co-location of *stx*, *eae*, and O serogroup would likely reduce this number.
- The dd-Check gave an additional eight samples that were presumptive positive and would have to go for culture confirmation, meaning the need for culture-based methods was not eliminated.

8.0 Recommendations

- Cost benefit analysis**:** Conduct a cost-benefit analysis to evaluate both direct costs, such as reagents, consumables, equipment, labour, and time, including the additional dilution steps required by dd-Check, and indirect costs, such as presumptive positives requiring confirmation and turnaround times. This should be compared against traditional screening methods to determine overall efficiency and financial viability.
- dd-Check Target Expansion: Enhance dd-Check by co-locating *stx*, *eae*, and O serogroups to address dynamic range limitations to improve confirmation reliability, and further the reduce the PP rate.
- Method Comparison Study**:** Encourage industries to trial dd-Check alongside existing screening methods to gain a comprehensive understanding of its benefits, including cost-effectiveness, turnaround time, and overall performance.

9.0 Project outputs

- Inception meeting held. Minutes of meeting provided to AMPC, Milestone 1 report submitted to, and approved by AMPC.
- Final Milestone report submitted to and approved by AMPC.

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11.0 Appendices

11.1 Appendix 1 Original Screen, IQ-Check, dd-Check and Culture Confirmation Data

dd#	Original PCR	IQ-Check	dd-Check	Culture Confirmed
dd#001	PP - O103, O111	PP - O103, O111	Positive linked targets	O111
dd#003	PP - O26	PP - O26	Negative: unlinked targets	
dd#004	PP - O26, O45, O157	PP - O45, O157	Positive: linked targets	O157
dd#005	PP - O26	PP - O26, O45	Negative	
dd#006	PP - O26	PP - O26	Negative: unlinked targets	
dd#007	PP - O103	PP - O103	Negative: unlinked targets	
dd#008	PP - O103, O157	PP - O103, O157	Positive: linked targets	O157
dd#010	PP - O26, O45, O121	PP - O26, O45, O121	Negative: unlinked targets	
dd#011	PP - O26, O45	PP - O26, O45, O121	Negative: unlinked targets	
dd#012	PP - O121	PP - O45, O121	Negative: unlinked targets	
dd#013	PP - O103	PP - O103	Negative: unlinked targets	
dd#014	PP - O45	PP - O45	Negative: unlinked targets	
dd#015	PP - O26, O45	PP - O45	Negative: unlinked targets	
dd#016	PP - O26, O45	PP - O26, O45	Negative: unlinked targets	
dd#017	PP - O26, O45, O103, O121	PP - O26, O45, O121	Negative: unlinked targets	
dd#019	PP - O103	PP - O103	Negative: unlinked targets	
dd#020	PP - O45, O103	O45, O103	Negative: unlinked targets	
dd#021	PP - O103, O111	PP - O103, O111	Positive: linked targets	O111
dd#022	PP - O103	PP - O45, O103	Positive: linked targets	
dd#024	PP - O26, O157	PP - O45, O157	Negative: unlinked targets	O157
dd#025	PP - O103	PP - O45, O103	Negative: unlinked targets	
dd#026	PP - O157	PP - O157	Positive: linked targets	O157

dd#027	PP - O45	PP - O45	Negative: unlinked targets	
dd#028	PP - O45, O103	PP - O45, O103	Negative: unlinked targets	
dd#030	PP - O26	PP - O26, O45	Negative	
dd#031	PP - O45, O121	PP - O26, O45, O121, O157	Positive: linked targets	
dd#032	PP - O45, O103, O121	PP - O26, O45, O121, O157	Negative: unlinked targets	
dd#033	PP - O26, O103	PP - O26, O103	Negative: unlinked targets	
dd#034	PP - O26	PP - O26	Presumptive positive	O26
dd#035	PP - O26	PP - O26	Negative: unlinked targets	
dd#036	PP - O26	PP - O26	Negative: unlinked targets	
dd#037	PP - O26, O45	PP - O26, O45	Negative: unlinked targets	
dd#038	PP - O26, O45	PP - O26, O45	Negative: unlinked targets	
dd#039	PP - O103, O121	PP - O103, O121	Negative: unlinked targets	
dd#041	PP - O103	PP - O103	Negative: unlinked targets	
dd#043	PP - O45, O157	PP - O45, O157	Positive: linked targets	O157
dd#044	PP - O157	PP - O103, O157	Positive: linked targets	O157
dd#046	PP - O26	PP - O26	Negative: unlinked targets	
dd#047	PP - O26, O103	PP - O26, O103	Negative: unlinked targets	
dd#048	PP - O26, O45, O121	PP - O26, O45, O121	Presumptive positive	
dd#049	PP - O26	PP - O26	Negative: unlinked targets	
dd#050	PP - O26, O121	PP - O26, O121	Presumptive positive	
dd#051	PP - O157	PP - O157	Positive: linked targets	O157
dd#053	PP - O157	PP - O157	Positive: linked targets	O157
dd#054	Not provided	PP - O26, O45, O103, O111	Negative: unlinked targets	O111
dd#055	Not provided	PP - O45, O121, O157	Positive: linked targets	O157
dd#057	Not provided	PP - O26, O45	Positive: linked targets	O26
dd#058	Not provided	PP - O45, O157	Positive: linked targets	O157
dd#059	Not provided	PP - O157	Positive: linked targets	O157
dd#066	PP - O45, O121	PP - O45, O121	Negative: unlinked targets	
dd#067	PP - O45	PP - O45	Negative	

dd#068	PP - O45	PP - O45	Negative	
dd#069	PP - O45	PP - O45	Negative: unlinked targets	
dd#071	PP - O145	PP - O145	Negative: unlinked targets	
dd#072	PP - O45	PP - O45, O121	Positive: linked targets	
dd#073	PP - O26	PP - O26	Negative	
dd#074	PP - O26	PP - O26, O45	Negative: unlinked targets	
dd#075	PP - O45, O121	PP - O45, O121	Negative	
dd#076	PP - O121	PP - O45, O121	Negative: unlinked targets	
dd#077	PP - O121	PP - O121	Negative	
dd#078	PP - O26, O45, O121	PP - O26, O45, O121	Negative: unlinked targets	
dd#079	PP - O26, O45	PP - O26, O45	Positive: linked targets	
dd#080	PP - O111	PP - O111	Positive: linked targets	O111
dd#081	Not provided	PP - O26	Positive: linked targets	O26
dd#082	PP – O103	PP - O26, O45, O103	Positive: linked targets	
dd#085	PP - O121	PP - O121	Negative	
dd#086	PP - O45	PP - O45	Presumptive positive	
dd#087	PP - O45, O157	PP - O45, O157	Positive: linked targets	
dd#088	PP - O26, O45, O103	PP - O26, O45	Positive: linked targets	
dd#090	PP - O26	PP - O26	Presumptive positive	
dd#091	PP - O26	PP - O26	Negative: unlinked targets	
dd#092	PP - O26	PP - O26	Negative: unlinked targets	
dd#093	PP - O26, O45, O103, O121	PP - O26, O45, O103, O121	Negative: unlinked targets	
dd#094	PP - O26, O45, O121	PP - O26, O45, O121	Negative: unlinked targets	
dd#095	PP - O26, O45	PP - O26, O45	Negative	
dd#096	PP - O157	PP - O157	Negative: unlinked targets	
dd#097	PP - O157	PP - O121	Positive: linked targets	
dd#100	Unknown	PP - O45, O103	Presumptive positive	
dd#101	PP - O103	PP - O45, O103	Negative	
dd#102	PP - O103, O111	PP - O103, O111	Negative: unlinked targets	

dd#106	PP - O157	PP - O45, O157	Presumptive positive	O157
dd#108	PP - O45	PP - O45	Negative: unlinked targets	
dd#109	PP - O45, O103	PP - O45	Negative: unlinked targets	
dd#110	PP - O45, O103, O121	PP - O103, O121	Negative: unlinked targets	
dd#111	PP - O103	PP - O103	Negative: unlinked targets	
dd#112	PP - O121	PP - O121	Negative: unlinked targets	
dd#113	PP - O26	PP - O26	Negative	
dd#114	PP - O26	PP - O26	Negative: unlinked targets	
dd#115	PP - O103, O121	PP - O103, O121	Negative	
dd#116	PP - O111	PP - O111	Presumptive positive	
dd#117	PP - O121	PP - O103, O121	Negative: unlinked targets	
dd#118	PP - O45	NA	Negative	
dd#119	PP - O45, O157	PP - O45	Negative: unlinked targets	
dd#120	PP - O103	PP - O103	Positive: linked targets	
dd#121	PP - O121	PP - O45, O121	Negative: unlinked targets	
dd#123	PP - O26, O103	PP - O26, O103	Negative: unlinked targets	
dd#125	PP - O45, O103	PP - O45, O103	Negative: unlinked targets	
dd#128	PP - O45, O103	PP - O45, O103	Negative: unlinked targets	
dd#129	PP - O45	PP - O45	Negative: unlinked targets	
dd#130	PP - O45, O103	PP - O45, O103	Negative: unlinked targets	