# Comparison Between Droplet Digital PCR (ddPCR) and qPCR for Screening and Confirming Shiga Toxin-Producing *E. coli* (STEC) with Linked and Unlinked *stx* and *eae* in Beef Matrices

BIO-RAD

## Introduction

Current qPCR-based methods for screening Shiga toxin-producing *Escherichia coli* (STEC) have the common challenge in differentiating between samples where a single organism contains both *stx* and *eae* virulence genes (true positive, linked virulence) from samples in which *stx* and *eae* reside in different organisms (false positive, unlinked virulence). Droplet Digital PCR (ddPCR) technology demonstrates the capacity in virulence linkage analysis by partitioning samples into nano-sized droplets containing intact cells where cell lysis and PCR amplification occur, enhancing the test accuracy by reducing false-positive reactions (Fig 1). The objective of this study (Fig. 2) was to evaluate the ability of the dd-Check STEC solution in detecting and distinguishing *E. coli* cells with linked and unlinked virulence compared to qPCR technology in three beef matrices.

**Fig. 1. Bacterial Encapsulation:** The Differentiator.

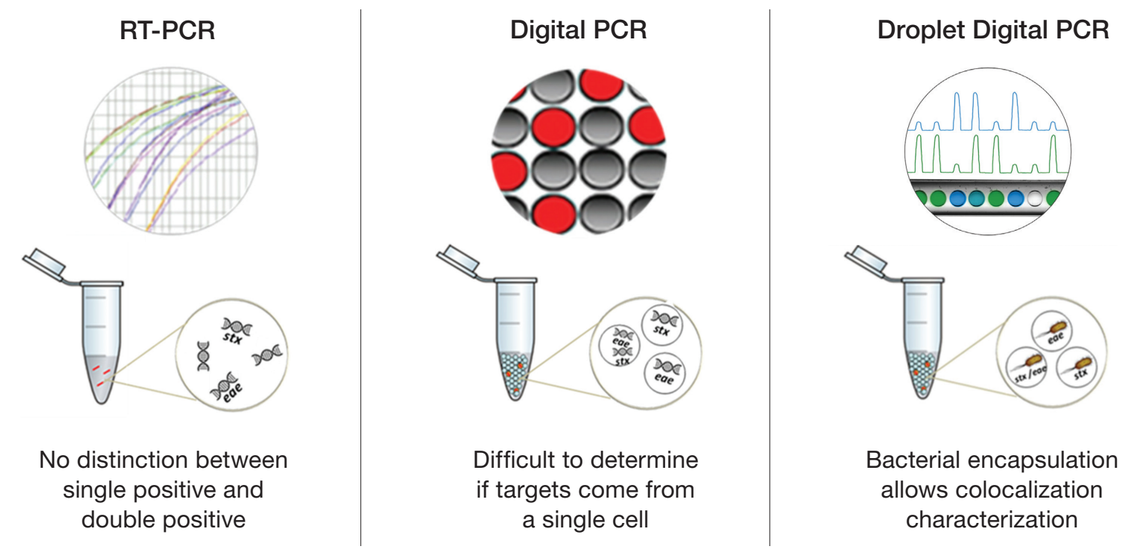



**Fig. 2. Study Objective and Experiment Design.**

### Objectives

**Improve STEC Testing Accuracy**

- Distinguishing between linked and unlinked *stx* and *eae*
- Reduce false positive

**Improve Time to Result and Flexibility**

- dd-Check STEC as a cultural-independent confirmation tool
- dd-Check STEC as primary screening tool

### Study Design

**Strain Selection**

- O157:H7 with linked *stx* and *eae*
- O45:H18 (*stx* only) + O45:H2 (*eae* only)

**Matrices**

- Ground beef
- Beef trim
- MicroTally

## Methods

A set of three matrices, beef trim, ground beef and MicroTally (n = 30), were inoculated at < 5 CFU per 375 g sample. For each sample type, 15 samples were inoculated with one regulated STEC strain with linked virulence genes (*stx* and *eae* in the same cell), and 15 samples were inoculated with a cocktail of two regulated serotypes with unlinked virulence genes (one with *stx* only and one with *eae* only).

All samples were processed following two timelines (Fig. 3) for evaluation of ddPCR technology as a primary screening assay compared to qPCR and as a confirmatory assay. Following the timeline for using ddPCR as primary screening, samples were processed for detection of *stx* and *eae* via qPCR (iQ-Check STEC VirX Kit, Bio-Rad) and ddPCR technologies (dd-Check STEC Solution, Bio-Rad) after a 16 hr enrichment. The workflow of ddPCR is illustrated in Fig. 4. Following the timeline for using ddPCR as a confirmatory assay, samples were processed for primary screening using qPCR after an 8 hr enrichment, followed by confirmation using ddPCR with or without regrowth according to the criteria demonstrated in Fig 3.

Cultural confirmation of STEC in all samples was performed following the USDA MLG 5C.00 protocol. The results obtained by qPCR and ddPCR assays were compared against the reference method for accuracy in screening and distinguishing STEC with linked and unlinked virulence genes.

**Fig. 3. Timeline for the Experiment Design.**

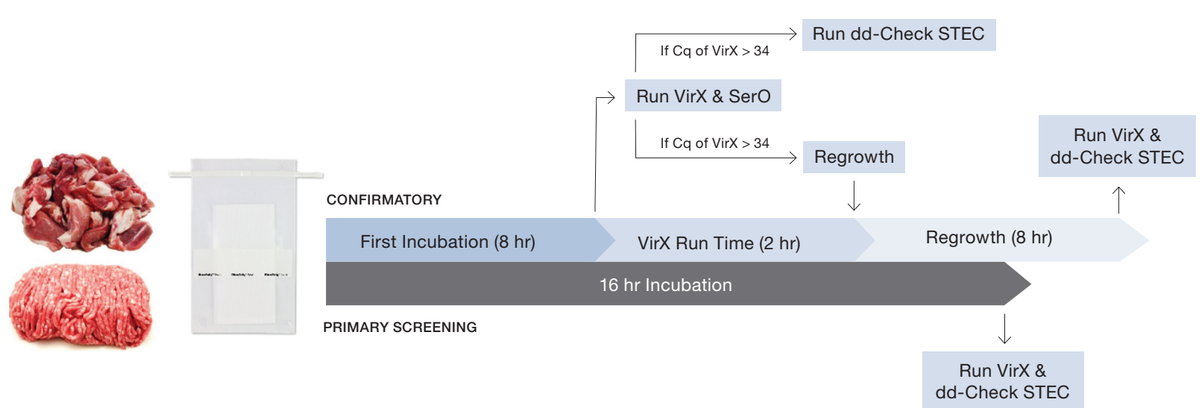
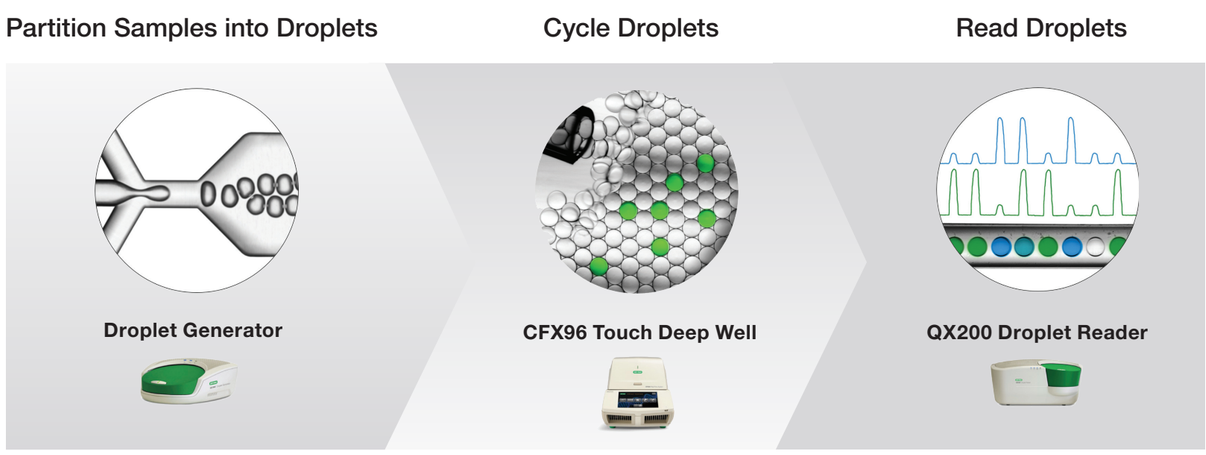



**Fig. 4. ddPCR Workflow.**




## Results

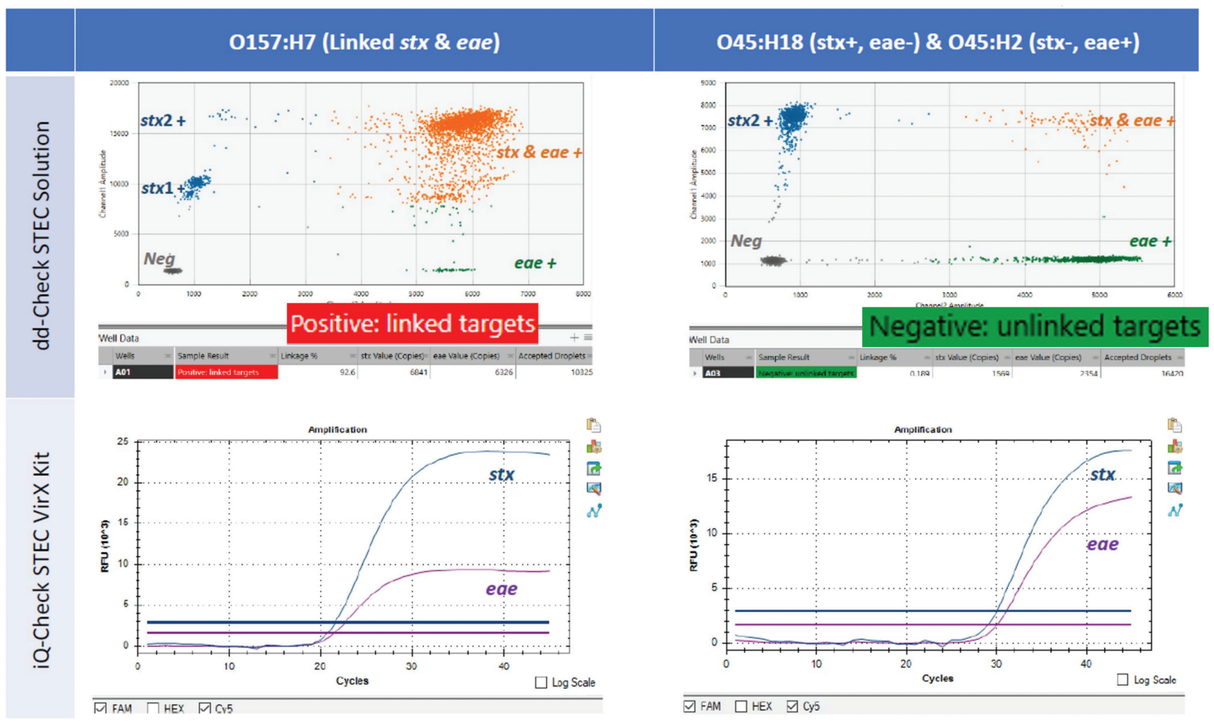
Results demonstrated that the ddPCR technology was able to individually identify the presence of *stx* and *eae* and verify the co-existence of these two virulence genes via linkage analysis (Table 1, Fig. 5).

**Table 1. Summary of results obtained from qPCR, ddPCR and cultural confirmation following the USDA MLG**

| Sample Type | Inoculum Type | 8 Hour VirX Screen (*stx* & *eae*) Positives | 16 Hour VirX (*stx* & *eae*) Positives | dd-Check STEC Linked Positives | USDA MLG Confirmed Positives |
|---|---|---|---|---|---|
| Beef Trim | O157:H7 (*stx*+/*eae*+) | 15/15 | 15/15 | 15/15 | 15/15 |
| | O45:H2 (*stx*-/*eae*+) O45:H18 (*stx*+/*eae*-) | 11/15[1] | 12/15[2] | 0/15 | 0/15 |
| Ground Beef | O157:H7 (*stx*+/*eae*+) | 15/15 | 15/15 | 15/15 | 15/15 |
| | O45:H2 (*stx*-/*eae*+) O45:H18 (*stx*+/*eae*-) | 15/15 | 15/15 | 0/15 | 0/15 |
| MicroTally | O157:H7 (*stx*+/*eae*+) | 15/15 | 15/15 | 15/15 | 15/15 |
| | O45:H2 (*stx*-/*eae*+) O45:H18 (*stx*+/*eae*-) | 14/15[3] | 14/15 | 0/15 | 0/15 |

[1] One sample was positive for *eae* only and three samples were positive for *stx*1/*stx*2 only.
[2] Three samples were positive for *stx*1/*stx*2 only.
[3] One sample was positive for *stx* only.

**Fig. 5. Comparison of data output between ddPCR and qPCR in distinguishing samples with linked and unlinked virulence targets.**




## Conclusion

- The dd-Check STEC assay was able to act as a screening tool to differentiate enrichments containing linked and unlinked *stx* and *eae* targets.
- The dd-Check STEC assay was also able to act as a culture independent confirmatory tool since all results were in alignment with USDA MLG cultural confirmation results.



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