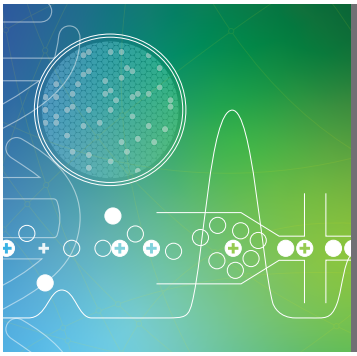

# dd-Check STEC Solution

## A Multipronged Approach to STEC Testing

### ddPCR Food Applications

- Microbial enumeration
- Virulence co-detection
- Food authenticity
- Parasites and viruses: Non-cultural targets

Current quantitative PCR (qPCR)-based methods for screening Shiga toxin-producing *Escherichia coli* (STEC) make it challenging to differentiate between samples in which a single organism contains both *stx* and *eae* genes (true positive, linked virulence) and samples in which *stx* and *eae* reside in different organisms (false positive, unlinked virulence).

Droplet Digital™ PCR (ddPCR™) technology demonstrates the capacity of virulence linkage analysis by partitioning intact cells into droplets in which cell lysis and PCR amplification occur, enhancing the screening accuracy by reducing false-positive reactions. With a high level of agreement in STEC confirmation between the dd-Check STEC Solution and the U.S. Department of Agriculture (USDA) MLG 5C.01 method, the dd-Check STEC Solution proves useful for culture-independent confirmation following primary screening by qPCR assays.

**A. Screening Method**


**B. Confirmatory Method**


**Fig. 1. Timelines for utilizing dd-Check STEC Solution as a screening and confirmatory method.** Follow the timeline for using ddPCR as a primary screening assay (**A**) and as a confirmatory assay (**B**).

* Presumptive positive samples from iQ-Check STEC VirX PCR Detection Kit should be tested on iQ-Check STEC SerO II Kit if serotype identification is required.

** Perform regrowth only if different media was used for screening or if iQ-Check STEC VirX Kit Cq is >34.

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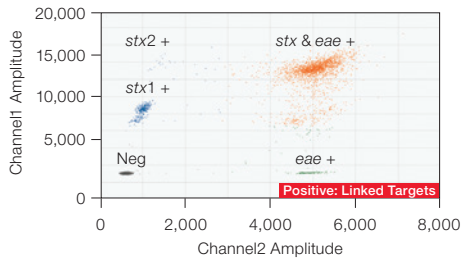
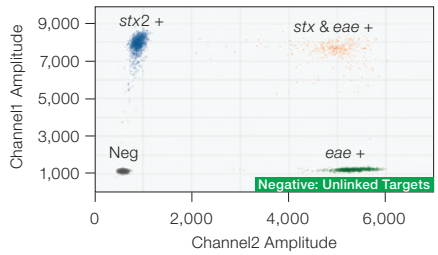
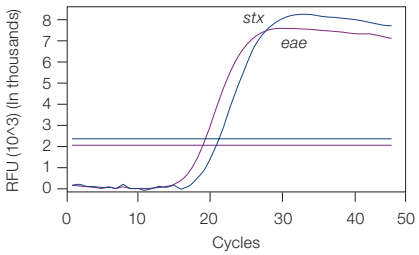
A. O157:H7 (linked *stx* and *eae*), tested with dd-Check STEC Solution




B. O45:H18 (*stx+ eae-*) and O45:H2 (*stx- eae+*), tested with dd-Check STEC Solution




C. O157:H7 (linked *stx* and *eae*), tested with iQ-Check STEC VirX Kit




D. O45:H18 (*stx+ eae-*) and O45:H2 (*stx- eae+*), tested with iQ-Check STEC VirX Kit

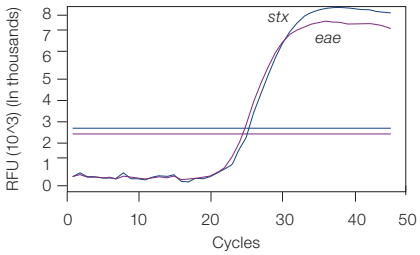



**Fig. 2. Comparison of data output between ddPCR and qPCR in distinguishing samples with linked and unlinked virulence targets.** This figure demonstrates that compared to the challenge of qPCR-based methods in differentiating sample enrichment with linked and unlinked targets, ddPCR technology was able to individually identify the presence of *stx* and *eae* to verify the co-existence of these two virulence genes via linkage analysis.

## Research Studies Evaluating dd-Check STEC as a Culture-Independent Confirmation Method for STEC in Beef Samples

Fresh and frozen enrichments from presumptive positive samples were processed for culture-independent confirmation using the dd-Check STEC Solution and cultural detection and confirmation following the USDA MLG 5C.01.

### Study 1: A major U.S. third-party food lab

**Study Objects**: 100 beef samples (beef trim and MicroTally) from a major beef producer in the U.S. that tested presumptive for *E. coli* O157:H7 via qPCR-based primary screening tests

#### Key Results

- For the confirmation of linked virulence profiles, the ddPCR assay and the USDA MLG 5C.01 identified the presence of linked targets in 97 and 95 samples, respectively, with 98% agreement
- Two of the four samples that were positive with the dd-Check STEC Solution but negative with MLG 5C.01 still produced colonies of STEC with *stx* and *eae*, but were negative with serogroup screening (Table 1)

**Table 1. Samples that tested positive and negative for STEC and MLG.**

| | Positive for MLG | Negative for MLG |
|---|---|---|
| **Positive for dd-Check STEC** | 93 | 4 |
| **Negative for dd-Check STEC** | 0 | 3 |

### Study 2: A government research lab

**Study Objects**: 100 beef samples (beef trim and MicroTally) that tested presumptive for *stx* and *eae* via iQ-Check STEC VirX Kit and one of the top 7 serogroups via iQ-Check STEC SerO II Kit

#### Key Results

- For the confirmation of linked virulence profiles, the ddPCR assay identified the presence of a linked target in the four samples that were initially culturally confirmed by the USDA Food Safety and Inspection Service (FSIS)
- Four samples that were positive with the dd-Check STEC Solution but negative with MLG 5C.01 still produced colonies of STEC with linked *stx* and *eae*, but were negative with serogroup screening (Table 2)

**Table 2. Samples that tested positive and negative for STEC and MLG.**

| | Positive for MLG | Negative for MLG |
|---|---|---|
| **Positive for dd-Check STEC** | 4 | 4 |
| **Negative for dd-Check STEC** | 0 | 92 |

Visit bio-rad.com/ddCheck for more information.



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***France*** *00 800 00 24 67 23* ***Germany*** *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300* ***Hungary*** *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050*
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***Russian Federation*** *00 800 00 24 67 23* ***Singapore*** *65 6415 3188* ***South Africa*** *00 800 00 24 67 23* ***Spain*** *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23*
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