



Paradigm Diagnostics STEC/Salmonella Broth (PDX-STE^C)

User Guide STEC/Salmonella Test Kit

Intended Use

Paradigm Diagnostics STEC Broth (PDX-STE^C) is intended for use in screening ground beef samples for the presence of viable Shiga toxin producing *E. coli* (STE^C) and *Salmonella* species. The medium is a selective enrichment designed to promote the growth of STE^C and *Salmonella* strains in the same enrichment broth. Following 18 hours of enrichment, crude broth samples can be streaked onto selective differential agars designed to detect and identify STE^C and *Salmonella* spp. Broth samples, optionally, can be processed to detect virulence genes for either STE^C or *Salmonella* via PCR analysis prior to plating onto selective differential agar.

Scientific Principal of the Test

PDX-STE^C contains selective agents to prevent the growth of competitive microflora while providing nutrients for growth of the resistant STE^C and *Salmonella*. *Salmonella* and STE^C are differentiated and identified by plating onto commercial selective, differential agar media such as CHROMagar™ and modified Rainbow® Agar products. STE^C positive colonies appear as mauve on CHROMagar™ STE^C, and *Salmonella* positive colonies appear as mauve colored colonies on CHROMagar™ *Salmonella* Plus. STE^C positive colonies appear as different shades from cream to mauve on Rainbow® agar. Isolated presumptive STE^C and *Salmonella* colonies can be confirmed using commercial lateral flow immunoassay tests for shiga toxins and *Salmonella* poly-O antigen. Colonies can also be confirmed using the appropriate probes and primers in qPCR analyses.

Diagnostic Performance Parameters

PDX-STE^C is comparable to the U. S. Department of Agriculture-Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (MLG) methods for *Salmonella* and shiga toxin producing *E. coli* for recovery of *Salmonella* and STE^C organisms.

Additional Notes

- Several commercial selective differential agar products are available. We recommend CHROMagar™ products because of their specificity.

Materials and Equipment Required

- An incubator capable of maintaining 32–44°C, such as an environmental cabinet, a water bath or a heating block.
- Stomacher™ bags, such as Cole Parmer. or equivalent.

Confirmation Step

Confirmation of presumptive positive samples can be carried out utilizing selective agars and biochemical tests such as those described in the most recent versions of the U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook methods for *Salmonella* and STEC.

Disposal

Decontaminate the PDX-SIB by autoclave is preferred. Bleach or other disinfectant can be used following a validated inactivation procedure in accordance with local, state and federal regulations.

Product Shelf Life

PDX-STECC currently has three months of shelf stability documented. The product should be stored at refrigerator temperatures (4°C) to obtain maximum useful life. PDX-STECC is packaged in 1L plastic bottles. It is available in alternative packaging upon request (e.g. 500 mL or 100 mL).

PDX-STECC is also available as a 1:10 liquid concentrate plus a single supplement. Sterile concentrate is supplied in 125mL plastic bottles which is added to 875 sterile water, one vial of supplement A to which is added 325g ground beef sample and stomached.

Precautions

1. Two non-STEC gram negative species were observed to grow in PDX-STEC: *C. braakii* and *E. cloacae*; contributing the presence of false positives.
2. Most *Salmonella* and STEC species are human pathogens and enriched samples should be handled employing Good Microbiological Practices.
3. All materials used should be handled and disposed of as potentially infectious material. Autoclaving is the preferred method of disposal. If autoclaving is not available, disinfectant solutions should be used; the disinfection protocol should be validated to inactivate the microorganisms.
4. Immuno-compromised individuals are particularly sensitive to infection by *Salmonella*, *E. coli* and should not be allowed in the vicinity of the testing.

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Instruction for PDX-STEC Use

1. Sampling: Place 325 g ground beef portions in 1.2 L capacity stomacher-type bags and massage for a few minutes to evenly distribute potential contaminants. Hold samples at room temperature for thirty minutes prior to addition of STEC selection medium. Add 1.0 L PDX-STEC to each bag and pummel for five minutes; **alternatively** add 125mL sterile liquid concentrate and one vial of supplement A and stomach ground beef in enrichment medium. Incubate bags at $42 \pm 1^\circ\text{C}$ for 18 to 24 hours. Streak 0.02 mL aliquots onto selective differential agars, such as CHROMagar™ STEC or modified Rainbow® agar for STEC and

CHROMagar™ Salmonella Plus agar or Xylose lysine Tergitol™ 4 agar for *Salmonella*. Optionally the enriched medium can be processed to purify genomic DNA for same-day identification of pathogens using the appropriate primers and probes for qPCR analyses.

2. Confirm mauve colonies for either STEC and/or *Salmonella* using commercial lateral flow immunoassay tests for shiga toxins (such as Certest Biotec) and Salmonella poly-O antigen (such as Salmonella Singlepath™). Genus confirmation for both *Salmonella* and *E. coli* can be conducted with a biochemical panel product such as Microgen GN-A™ or similar products.
3. Alternative identification can be accomplished using purified DNA from colony isolates as template DNA in PCR amplification analyses with the appropriate accompanying primers and probes.