# Top 7 STEC Flowchart
## (*E.coli* O157:H7, O26, O45, O103, O111, O121, O145)

## SAMPLE & EREB ENRICHMENT

Raw Beef Trim
375 g

Raw Ground Beef
375 g

Enrich with 1 L ± 50 mL EREB
Incubate at 42°C ± 1°C for 10-18 hours
(12-18 hours if pooled lysis)

## SAMPLE LYSIS

**5 µL**
Sample

**400 µL**
Lysis Buffer

**95°C ± 3°C**

10 min

5 min

Room
Temperature

**OPTIONAL** - Pooled Lysate
Pool up to 10 lysates by combining 5 µL of each lysate and pipette mix

## SET-UP SIMUL-QPCR ASSAY

**20 µL**

Place strips in
PCR and run

## REVIEW SIMUL-QPCR ASSAY

**Results** within 1 hour

No $C_q$
=
**Negative**

$C_q$ with typical
amplification /
sigmoidal curve
=
**Positive**

## AOAC PTM WORKFLOW CONCLUSION

| | TARGET INTERPRETATION |
|---|---|
| ***E. coli* O157:H7 Assay** | *E. coli* O157:H7 |
| **Shiga Toxin (stx) and Intimin (eae) Assay** | Shiga Toxin (stx) |
| | Intimin (eae) |
| ***E. coli* O26 and *E. coli* O103 Assay** | *E. coli* O26 |
| | *E. coli* O103 |
| ***E. coli* O111 and *E. coli* O145 Assay** | *E. coli* O111 |
| | *E. coli* O145 |
| ***E. coli* O45 and *E. coli* O121 Assay** | *E. coli* O45 |
| | *E. coli* O121 |

PERFORMANCE TESTED
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