Validation of the Hygiena™ BAX® System Real-Time PCR Assays for *Salmonella*, *E. coli* O157:H7, and STEC for Dried Cannabis Flower and Dried Hemp Flower

This matrix extension study was conducted under the AOAC® *Performance Tested Methods*^{SM} *(PTM)* program, the *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces,* SMPR 2020.002 and SMPR 2020.012. The independent laboratory study was conducted by TEQ Analytical Laboratories, Inc. (Aurora, CO).

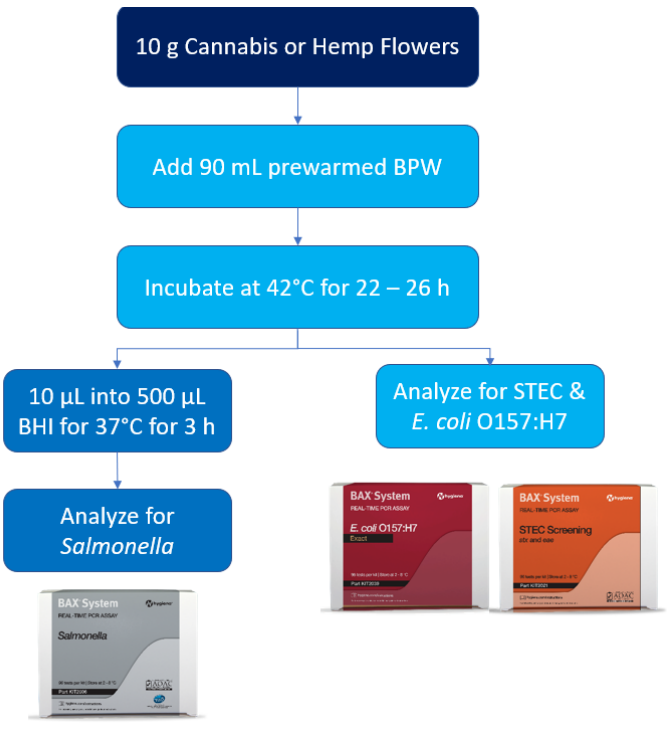
Validation Methods

- Validation of the alternative method was performed, and results confirmed by following SMPR 2020.002 and SMPR 2020.012 guidance recommendations:
  - Dried Cannabis Flower (> 0.3% THC) – 10 g
  - Dried Hemp Flower (≤ 0.3% THC) – 10 g
- Enrichment Conditions*:
  - *E. coli* O157:H7 and STEC:
    - Add 90 mL of pre-warmed (37-42°C) Buffered Peptone Water (BPW), incubate at 42 ± 1°C for 22 h - 26 h
  - *Salmonella*:
    - Add 90 mL of pre-warmed (37-42°C) BPW; incubate at 42 ± 1°C for 22 h - 26 h
    - Add 10 µL enrichment to 500 µL pre-warmed (37°C) BHI Broth and incubate at 37 ± 1°C for 3 h
  - Following incubation, DNA extraction was performed with BAX® System lysis methods for real-time PCR; lysates were analyzed by BAX® System Real-time PCR Assays for *Salmonella* (KIT2006), *E. coli* O157:H7 EXACT (KIT2029), and STEC Screening (KIT2021).

*Note: Smaller sample sizes (e.g. 1 g) can be utilized as long as the 1:10 matrix to media ratio is maintained and all other enrichment steps are followed.

Validation Results

- The level 2 modification validation studies (AOAC Certifications: 081201, 102003, 090301) indicated that the evaluation consisted of matrix studies to extend the method claim to include dried cannabis flower and dried hemp flower.
- Results of the validation study demonstrated performance of the BAX® System assays in accordance with Standard Method Performance Requirements (AOAC SMPR 2020.002 and SMPR 2020.012)
- Additionally, the acceptability limits for the sensitivity and the Relative LOD studies (1 CFU/Sample) for all matrices and enrichment protocols were met.



Industry Significance

- This validation provides a single enrichment option for cannabis and hemp flowers that is robust and reliable for the detection of *Salmonella*, STEC, and *E. coli* O157:H7 while also being easy to utilize in high-throughput laboratories.