AOAC Performance Tested Methods℠ — Draft Validation Study Plan

Method LUME-1: Continuous In-Situ TLF Screen for Fecal Contamination in Recreational Water

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Status. A *draft* study outline to seed the AOAC RI consulting phase, in which the developer and an AOAC RI technical consultant jointly finalize the validation outline. It maps the PTM / ISO 16140-2 study elements onto the Lume and identifies which existing data can be credited and which studies must be run. Numeric acceptance criteria marked `[TBD]` are finalized with AOAC RI from the study data.

Contents

1.0 Scope and Certification Claim

1.1 **Method.** Method LUME-1: continuous, in-situ tryptophan-like fluorescence (TLF) screen for fecal contamination, with per-unit calibration and temperature/turbidity corrections (see the method document).

1.2 **Claim.** Screening classification of *E. coli* exceedance at an applicable recreational action limit (primary: 126 CFU/100 mL) against an EPA reference method; continuous quantitative fecal-contamination index as a secondary output.

1.3 **Matrices.** Ambient recreational fresh water (rivers, streams, lakes); marine/estuarine as a planned claim extension.

1.4 **Validation framework.** Harmonized with ISO 16140-2 and the AOAC RI microbiological (and, where applicable, quantitative-method) validation guidelines.

2.0 Reference Methods and Matrices

2.1 **Reference method.** IDEXX Colilert/Quanti-Tray/2000 (defined-substrate, MPN) and/or EPA Method 1603 (membrane filtration, CFU), as designated by AOAC RI.

2.2 **Reference measurement uncertainty.** The reference is treated as a value with its own uncertainty (Quanti-Tray 95% CI); the two EPA methods are not equivalent to each other (ISO 17994). Evaluation reports both point-labelled and uncertainty-propagated performance.

2.3 **Matrices.** A range of recreational freshwater matrices spanning turbidity and temperature, including at least one high-turbidity matrix and wet/dry/storm/bloom conditions.

3.0 Study Design Overview

Two studies per the PTM model:

3.1 **Developer (single-laboratory) validation.** Conducted by Virridy per this plan; establishes method-comparison, matrix, selectivity, robustness, reproducibility, and stability performance.

3.2 **Independent-laboratory validation.** An AOAC-RI-selected independent laboratory reproduces the core method-comparison and confirms performance against the reference under an AOAC study director / expert reviewer.

4.0 Performance Characteristics and Studies

4.1 Method comparison / relative accuracy

Paired sensor-vs-reference samples across the working range.

- **Quantitative.** Regression and Bland–Altman limits of agreement on $\log_{10}$ concentration; Willmott Index of Agreement (EPA AltCalc). *Existing:* IA 0.96 (Colilert, $n = 209$), 0.91 (MF, $n = 206$); R^2 0.86 / 0.70; LoA $[-0.42, +0.42]$ vs. Colilert.
- **Threshold classification (screening).** Sensitivity, specificity, and (POD-style) probability of detection at the action limit. *Existing:* at 126 CFU/100 mL vs. Colilert, sensitivity 0.94, specificity 0.86, balanced accuracy 0.90 (screening operating point).

4.2 Matrix study

Performance across multiple recreational freshwater matrices and conditions; per-matrix agreement and classification. *Existing:* 661 paired samples across 12 sites (US + Europe), including the independent Seine/Marne deployment (96.8% accuracy at the 900 CFU threshold).

4.3 Selectivity and interferences (TLF analogue of inclusivity/exclusivity)

Because TLF is not organism-specific, "selectivity" is characterized as response to the target signal versus documented interferents rather than strain inclusivity/exclusivity:

- Dissolved/humic-like organic matter (fDOM); turbidity; temperature quench — the three interferences the method corrects for.
- Free chlorine attenuation (relevant to treated-water controls).

4.4 Robustness

Deliberate variation of operating conditions within the validated envelope: temperature range, turbidity range, and the per-unit correction parameters; confirm the corrections hold. Ties to the controlled temperature/turbidity series in the per-unit conformance procedure.

4.5 Inter-unit reproducibility and lot-to-lot

For a per-unit-calibrated device, "lot-to-lot" maps to **unit-to-unit** reproducibility: co-located units reading a common water; paired $\log_{10}$ difference. *Existing:* inter-unit $\sigma \approx 0.16$ $\log_{10}$ — the primary gating item; the study targets reduction toward the reference (≈ 0.10 $\log_{10}$).

4.6 Stability and calibration verification

Baseline/calibration drift over a deployment interval, biofouling diagnostics, and continuing-calibration verification (QCS), per the per-unit conformance certificate. Confirms results remain in-specification in service.

5.0 Acceptance Criteria

Acceptance criteria shall not be looser than the validated performance of Method LUME-1; finalized with AOAC RI from the study distributions.

Characteristic	Draft acceptance target
Agreement vs. reference (IA)	IA ≥ 0.70 (EPA AltCalc); *achieved: 0.91–0.96*
Regression / limits of agreement	LoA within `[TBD]` $\log_{10}$; *achieved:* ± 0.42 *vs. Colilert*
Exceedance classification	sensitivity / specificity $\geq$ `[TBD]`; *achieved: 0.94 / 0.86 at 126*
Matrix consistency	claim met in each matrix (`[TBD]` matrices)
Inter-unit reproducibility	paired difference $\leq$ `[TBD]` $\log_{10}$ (target $\approx$ 0.10)
Robustness	corrections hold across the temperature/turbidity envelope
Stability / drift	within `[TBD]` over `[TBD]` interval

6.0 Reuse of Existing Data

Much of the developer study is already in hand and can be proposed for credit toward the PTM validation:

Study element	Existing evidence
Method comparison (quantitative)	IA 0.96 / 0.91; R^2 0.86 / 0.70; LoA ± 0.42; $n = 209/206$
Threshold classification	126 CFU: sens 0.94 / spec 0.86 / BA 0.90 (vs. Colilert); 0.97 / 0.99 / 0.98 (vs. MF)
Matrix / field breadth	661 paired samples, 12 sites; Seine 96.8% @ 900 CFU
Reference uncertainty	Quanti-Tray 95% CI propagation; ISO 17994 reference non-equivalence
Inter-unit reproducibility	$\sigma \approx 0.16$ $\log_{10}$ (to be improved)
Selectivity / interferences	documented temperature, turbidity, DOM, chlorine responses (Method LUME-1 §4)
Stability / QC	per-unit conformance certificate procedures

7.0 Open Items

8.1 Governing validation track (quantitative vs. qualitative/screening) — AOAC RI to select.

8.2 Reference method designation (Colilert vs. 1603/MF) — align with EPA pathway; resolve the S-Endo vs. 1603 MF question.

8.3 Inter-unit reproducibility target and demonstration.

8.4 Matrix count and independent-lab scope.

8.5 Marine/enterococci claim extension timing.

8.0 References

[1] ISO 16140-2, *Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.*

[2] AOAC INTERNATIONAL, *Performance Tested Methods^{SM} Program* (AOAC Research Institute).

[3] U.S. EPA, *Site-Specific Alternative Recreational Criteria: Alternative Indicators and Methods* (Technical Support Materials) and Alternative Methods Calculator.

[4] U.S. EPA Method 1603; IDEXX Colilert/Quanti-Tray.

[5] ISO 17994, *Water quality — Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods.*