

# Review of monitoring techniques and sampling strategies to identify the most significant sources of faecal indicator organisms (FIO) within a catchment

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# Executive Summary

## Questions

1. Which current monitoring technology has been used successfully as part of field investigations to identify major sources of Faecal Indicator Organisms (FIO) within catchments, i.e. FIO hotspots?
2. Which emerging technologies are likely to be applicable and practical for identifying FIO hotspots?
3. How often would Scottish Environment Protection Agency (SEPA) need to sample to identify FIO hotspots and verify FIO modelled exports given storage and monitoring resource limitations in remote areas and complex river networks<sup>1</sup>?
4. What monitoring strategy would SEPA need to apply to address the pressure profile within the area of influence (aka zone of influence)?<sup>2</sup>
5. What is the timing of FIO discharges expected from each FIO source type, e.g. septic tank systems (STS), combined sewage overflows (CSO), storm-tank overflows (STO), wastewater treatment works (WwTW), wildlife and farmland?

## Background

SEPA plan to use “blitz” monitoring to get a picture of water quality across catchments where there are multiple sources of faecal pollution to Bathing Water Protected Areas (BWPA) and Shellfish Water Protected Areas (SWPA). This is envisaged to involve FIO sampling across the river network to identify the area of influence, and trace FIO hotspots and types of sources within the area of influence. However, blitz monitoring is faced with a wide range of challenges, such as monitoring resource limitations, regulatory requirements for storage time and analytical procedures, and limited understanding where the area of influence and FIO hotspots are located. Addressing these challenges is essential for addressing the impacts of catchment-based faecal pollution to BWPA and SWPA.

## Research undertaken

We undertook a literature review summarising best available evidence on the timing of FIO discharges, in-stream FIO variability, FIO pollution risk, FIO monitoring and detection technologies. We developed a desktop approach to identifying potential FIO hotspots. We also developed recommendations for a practical monitoring

<sup>1</sup> No source-apportionment needed.

<sup>2</sup> Here, this refers to the part of the river catchment in which diffuse and point FIO pollution sources can influence water quality in bathing water protected areas (BWPA) and shellfish water protected areas (SWPA).

strategy to identify the area of influence to BWPA and SWPA, and to track FIO from different FIO hotspots and types of sources within it.

## Key findings

- There is sufficient understanding of the broad factors determining timing of FIO discharges from different types of sources, in-stream FIO variation and FIO pollution risk across a river catchment.
- There is consensus among experts on the monitoring strategy needed to identify the area of influence and FIO hotspots therein as well as to differentiate between types of sources (i.e. human vs animal) within the area of influence.
- Current FIO technologies successfully used for FIO catchment investigations in the lab include cultivation-based methods (e.g. membrane filtration, Coliscan Easy gel system and Colilert); DNA-based methods (e.g. qPCR); biomarkers (e.g. sterols); or chemical tracers (e.g. caffeine, saccharin).
- Current FIO technologies successfully used for FIO catchment investigations in the field include using mobile labs after sample collection (without storage time) (e.g. Aquaflex and Colitag) and probes for proxy measurements (e.g. turbidity, conductivity, ammonia and temperature), or using *in situ* devices for continuous measurements (autosamplers).
- There is limited published information for the use of emerging FIO technologies in FIO catchment investigations. Technologies that could possibly be applied include:
  - o In the lab after field sample collection (e.g. RNA biosensors, Flow cytometry and Fluorescent Activated Cell Sorting, Paper-Origami DNA microfluidics and DNA-based methods for microbial source tracking (MST) such as microarray).
  - o In the field, probes (e.g. Bacti-Wader, aquaCHECK365, Bactiquant Water, Microbial Bioanalyser), or continuous monitoring technologies based on the detection of enzymatic activities (e.g. BACTcontrol).
  - o Emerging technologies are most powerful when used in combination with current technologies (e.g. aquaCHECK365 applied in combination with Colitag or turbidity sampling).
- Frequency of sampling for a given current or emerging FIO technology depends on the purpose of sampling and knowledge of in-stream FIO variability at different scales at a given site and time. However, sampling frequency per FIO technology remains briefly addressed in the literature.

- The monitoring strategy to detecting catchment-based FIO sources involves three phases:
  - o Phase 1 identifies the area of influence and FIO hotspots therein through field surveys and monitoring with a desk-based initial screening component in data-rich catchments.
  - o Phase 2 studies in-stream FIO variability in relation to rainfall-dependent/-independent discharges from FIO hotspots in the area of influence through monitoring and modelling.
  - o Phase 3 involves monitoring in the area of influence to elucidate predominant types (i.e. human vs animals) of diffuse FIO sources using microbial and chemical source tracking tools.
- FIO discharges may be rainfall-dependent (e.g. CSO, STO and farmland runoff) or rainfall-independent (e.g. WwTW and STS effluent, artificial drains, livestock, wildlife, and leaching from STS soakaways).
- Temporal variability of in-stream FIO concentrations may be diurnal, storm event-scale, seasonal and interannual.
- Inspect area for FIO risk from unmapped STS, wildlife, pets and other diffuse sources (e.g. streambed and streambanks) and verify their inputs.
- Select sampling sites that clearly link to known FIO sources, are wildlife-free when sampling, and display small variability during baseflow.
- Collect samples in short periods of time during wet and dry conditions (hybrid monitoring design) to address variability from rainfall-dependent and rainfall-independent discharges.

3. Identify the upstream limit of FIO pollution through monitoring upstream and downstream (“bracketing”) potential FIO hotspots until a “clean” sample indicates no FIO impact from upstream. The area of influence may be sought upstream from the coastal waterbody catchments.

**Phase 2: Apply membrane filtration techniques and flow cytometry in the lab or use mobile labs (e.g. Colitag) or continuous monitoring devices (e.g. ALERT – *E. coli* Analyser) to assess temporal variability of in-stream FIO (area of influence) concurrently with turbidity, temperature and flow.**

Monitoring can be:

- Hourly for a day or two upstream and downstream of continuous human (e.g. WwTW and STS clusters) and/or animal (e.g. livestock farmland) FIO discharges during wet and dry days.
- Weekly or twice weekly (bi-weekly) for as long as necessary to understand discharges from CSO, STS clusters, and stream-river confluence sites.
- Event-scale to study the effects of rainfall-dependent FIO discharges such as CSO, STO and farmland runoff. Event-scale data can be redrawn from weekly time series.

**Phase 3: Apply microarray, qPCR of genetic markers or flow cytometry for MST to track predominant FIO sources at sites influenced by diffuse sources or mixed land use.** This sampling is confirmatory or hypothesis-driven based on the evidence from Phase 1 and 2 on in-stream FIO variability downstream of CSO, STS clusters, confluence sites and at BWPA/SWPA. Sampling for MST can target wet and dry conditions or be one-off.

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## Recommendations<sup>3</sup>

### Phase 1: Apply a toolbox approach integrating desktop studies, field monitoring and modelling:

1. Use the desktop screening approach developed here to identify potential FIO hotspots, e.g.:
  - Point sources such as CSO, STO, WwTW serving more than 5000 people or tourist resorts; high-density STS clusters (>20 STS/km<sup>2</sup>) and STS within 10 to 50m from watercourses located on soils at high runoff risk/leaching potential.
  - Diffuse sources including modelled areas of high in-stream FIO risk from livestock
2. Apply mobile lab technologies such as Colitag and aquaCHECK365 in combination with turbidity<sup>4</sup>, temperature and flow to verify locations and FIO pollution from each potential FIO hotspot identified in the desktop study, as follows:
  - Start from the waterbody catchments adjacent to BWPA or SWPA (i.e. coastal catchments).
  - Prioritise human FIO hotspots (i.e. CSO, WwTW, STO, STS) or stream-river confluence sites draining areas influenced by human FIO sources in the coastal waterbody catchments.

<sup>3</sup> Our recommendations regarding the choice of monitoring technologies are based on the requirements from SEPA and do not preclude the use of the technologies that were not recommended for other purposes.

<sup>4</sup> For detecting wastewater downstream of point sources and not as a surrogate for FIO, unless preliminary data suggest that turbidity correlates significantly with levels of FIO in the study area.