Field Sampling Guide for Molecular Water Quality Analyses

This guide discusses best-practice recommendations for collecting water samples that will be evaluated using molecular methods (e.g., ddPCR, qPCR, LAMP, NGS). It is designed for field crews, consultants, and utility staff who require defensible data for microbial source tracking, pathogen surveillance, antibiotic-resistance or eDNA monitoring.

1. Planning

- Clear objectives help determine site selection, frequency, volume, and QA/QC requirements.
- Review drainage maps, sewer overlays, land-use data, and historical water-quality records. Conduct a site walk to confirm access, safety, and flow conditions.
- Determine sites, event triggers, QA/QC elements, and holding-times.
- Refer to Digital MIQE, EMMI, and relevant regulatory guidance.

2. Site Selection

- Examples of targeted sites include outfalls, storm drains, tile drains, marinas, agricultural runoff channels, and upstream/downstream of livestock areas.
- For reference or background sites target locations with minimal human influence to establish baseline conditions.
- Ensure the collection point connects with upstream flow; avoid isolated pools or backwater eddies.
- Secure right-of-entry, obtain permits for protected or tribal lands, and coordinate with facility operators for restricted areas.

3. Preventing Cross-Contamination

- Wear new gloves at each site.
- Decontaminate poles, bailers, and filtration rigs with 10 % bleach; rinse with site water, then DNA-free water.
- Use different gear for high-signal sites (e.g., raw wastewater) and low-signal sites (e.g., drinking water sources).

4. Representativeness

- Where should I sample?
  - Well-mixed point: If tributary flows are blended mid-channel or at a header/manhole, one grab is representative of the whole drainage area.
  - Wide or stratified waters: If the channel or pipe is >1 m across or shows layering, create a composite to average variability by combining grabs from left-center-right or top-mid-bottom.
  - Context site: When source attribution matters, pair the representative site with an upstream/background location (or influent/effluent taps) to isolate the target signal.
- When should I sample?
  - Dry-weather baseflow: captures chronic leaks or failing infrastructure.
  - Wet-weather/first-flush (0–60 min): captures episodic, runoff-driven loads.
  - Diurnal peaks: human sewage often strongest 06:00–10:00.
  - Flow & Tide: Use a consistent tidal stage when trend-monitoring estuaries. Record stage, discharge (if gauged), and tide phase.

5. **Sample Collection**
    - Volume
        - Collect 250 mL to 1 L.
        - High-turbidity or low-biomass matrices may require ≥2 L or in-field filtration. To increase sensitivity of targets in low concentrations, consider concentrating ≥100 L via hollow-fiber ultrafiltration.

    - Containers & Preservatives
        - Sterile polypropylene or HDPE, wide-mouth, DNA-free.
        - Add sodium thiosulfate when sampling chlorinated effluents.

    - Field Filtration (optional)
        - 0.45 µm or 0.22 µm membranes or electronegative cartridges for eDNA/pathogen capture.
        - Record filtered volume.

    - Technique
        - Face the bottle upstream; uncap immediately before submersion.
        - Use telescoping poles if wading is unsafe; avoid disturbing sediments.
        - Collect mid-channel at 10–15 cm depth
        - Avoid shoreline scum, sediment plumes, or surface foams unless specifically targeted.

    - Replicates & QA/QC
        - Collect field duplicates (~10 %) to assess precision.
        - Include at least one blank (equipment or trip) per 10 environmental samples or per sampling day, whichever is greater, to detect sampling contamination.

    - Documentation
        - Include sample ID, date, time, site location, and sampler initials on both the sample label and chain of custody.
        - Record any field observations or metadata that is critical for the project.

6. **Handling, Preservation & Transport**
    - Place samples on ice or store around 4°C immediately.
    - Ship or hand-deliver samples in a sealed cooler with ice packs. Aim for the laboratory to receive them within 10 hours if possible, and within 24 hours when testing for pathogens. Overnight shipping is recommended.
    - If holding times will exceed 24 hours, perform filtration in the field and freeze the filters at -20°C or colder. Ship them overnight on dry ice.
    - Ensure that a completed CoC accompanies the samples and apply tamper-evident seals for regulatory projects.

7. **Special Considerations**
    - Storm Event Automation – To collect time- or flow-weighted composites, deploy autosamplers with turbidity or rainfall triggers.
    - High-Turbidity/Particle-Rich Waters - Pre-filter coarse particles on-site to minimize PCR inhibitors and filter clogging; keep samples chilled and shaded until processing.

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