



NATIONAL INSTITUTES OF HEALTH, PAKISTAN

Wastewater Environmental Surveillance (WES) Programme

STANDARD OPERATING PROCEDURES

Wastewater Grab Sample Collection, Labeling & Cold-Chain Transport

PREPARED BY

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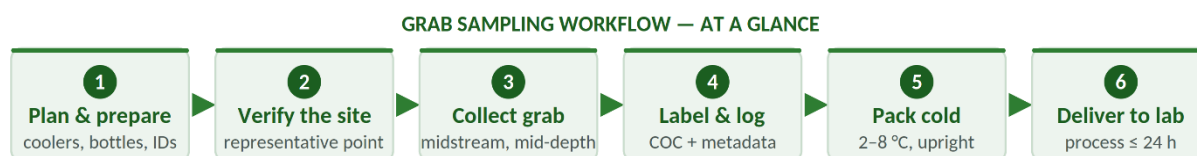
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1 Purpose

Provide clear, reproducible steps for field personnel to collect representative **wastewater grab samples** from open drains, closed drains and pumping stations for microbiological and molecular (DNA/RNA) analyses, and to label, document, preserve and transport them using an appropriate cold chain so sample integrity is maintained for laboratory processing.



Six stages every grab sample passes through, from planning to laboratory receipt.

2 Scope

This SOP applies to all field staff collecting wastewater grab samples for environmental surveillance, pathogen testing (viral, bacterial, fungal), or research in Pakistan (urban sewer networks, open drains, pumping stations, community sewers, septic outfalls).

Note: This SOP aligns with national and international guidance (EPA, CDC, WHO, APHL, Standard Methods)

3 Definitions

Grab sample — A single discrete sample collected at a specific time (or discrete samples collected within ≤15 minutes) that represents conditions at that time.

Cold chain — Containment plus temperature control (ice packs/cooler and, ideally, a data logger if available) used to keep samples at 2 to 8 °C from collection until laboratory receipt.

4 Responsibilities

- **Field Supervision:** site selection confirmation, safety brief, final COC sign-off, cooler prep oversight.
- **Sampler(s):** perform sampling, fill labels/COC, and maintain custody, document environmental metadata (time, weather, flow characteristics).
- **Transporter / Courier:** maintain cooler temperature during transport, hand samples to receiving lab and get receiver signature.
- **Receiving Lab:** check chain-of-custody, record sample temp on arrival, refrigerate or process.

5 Safety & PPE

- **Standard PPE:** nitrile gloves (change between sites), eye protection, impermeable splash apron or coveralls, rubber boots, surgical or N95 mask (site risk dependent), hard hat where required. Perform site-specific risk assessment (traffic, contaminated surfaces, confined space).
- **Avoid leaning over open channels;** use sampling pole or bucket with rope; never reach into flow by hand. Follow local occupational safety rules.
- **Decontaminate equipment and surfaces after sampling with appropriate disinfectants;** dispose of used PPE per local regulations. (Follow BSL/field safety guidance; treat wastewater as potentially infectious).



For the reference please view: https://drive.google.com/file/d/1f5XmMOVawyZYFmmtmAauP2_C9yqh-A9a/view?usp=drive_link

6 Equipment & Consumables Checklist

- Sterile sample bottles for microbiological testing (200 mL for WES project); pre-labeled if possible (polypropylene or PET, sterile).
- Sampling pole and rope with sterile bottle holder or sterile sampling bucket (long pole for open drains).
- Cooler boxes with ice packs (pre-frozen) and insulating material.
- A temperature monitor or a data logger, or an electronic thermometer to record cooler temperature on receipt.
- Chain-of-custody (COC) form and field logbook. Printable sample labels and waterproof marker.
- Personal protective equipment (gloves, goggles, boots, aprons/overalls).
- Decontamination supplies (70% isopropanol or chlorine solution, disposable wipes).
- Secondary containment bags (zip bags) and tamper-evident seals.
- Hand sanitizer and first aid kit.
- Field flow meter or staff gauge (optional, can be useful for metadata collection).



For the reference please view: https://drive.google.com/file/d/1f5XmMOVawyZYFmmtmAauP2_C9yqh-A9a/view?usp=drive_link

7 Site Selection: Representative Sampling Points

When choosing the exact point to collect a grab sample, aim for a location that best represents the sewer catchment and avoids atypical disturbances (e.g., industrial discharges). Confirm physical access and safety with local utility staff where possible.

Open drains (urban)

- Midstream, where flow is well established (avoid stagnant pools and eddies — an eddy is the swirling of water and the reverse current created when the water is in a turbulent flow regime). Ideally, at a straight reach of 5 to 10 m downstream of bends or inflows. Collect from the centerline of flow or use a pole to reach mid-flow.
- Avoid collecting direct stormwater or industrial effluent inlets unless those are the surveillance target. Document nearby sources as well.

Covered / closed drains (sewer manholes)

- Sample from the influent channel inside a manhole: at the lowest point where flow concentrates, but avoid sampling right at the vertical drop. Use a sampling pole or bailer. Note whether access requires confined space precautions.
- If flow is intermittent, target periods of highest flow (morning peak) to improve representativeness.

Pumping stations and influent to treatment works

- Sample at the station's influent (before pumps or chemical addition) to represent the upstream catchment. For pump stations, sample in the wet well at mid-depth if safe or at an influent channel. Coordinate with plant staff to avoid sampling during mechanical operations that cause atypical mixing.

Practical site checklist — site ID points to record

1. Site name (unique), GPS coordinates (lat/long), nearest landmark.

2. Type: open drain / closed drain (manhole) / pumping station / WWTP influent.
3. Upstream/downstream landmarks, approximate catchment population (if known).
4. Date & time (24-hr), sampler name/initials.
5. Flow condition — flowing / slow / stagnant and approximate flow (m³/s or visual).
6. Weather (rain in the last 24 h?), recent discharge events.

8 Sampling Frequency & Timing

Grab sampling captures one moment, so be consistent about time of day across repeated surveys for comparability (e.g., daily morning peak 06:00–09:00). CDC recommends consistent scheduling to reduce variability.

9 Step-by-Step Grab Sampling Procedure

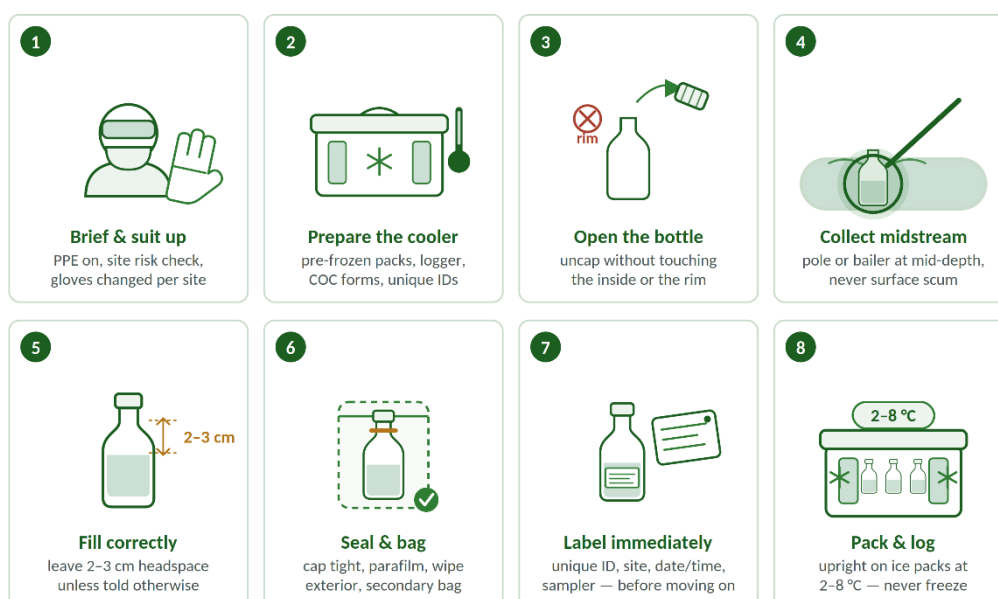
Before leaving base

1. Verify sample request and required volume with the lab (WES project analytes: qPCR and sequencing).
2. Confirm container type and whether preservatives are required (WES project: polypropylene-based sterile container and no preservative for the cold-chain logistics stage).
3. Prepare coolers: freeze ice packs overnight; set aside waterproof labels/markers, COC forms and thermometer or data logger. Document cooler ID.
4. Inspect sterile bottles (expiry/sterility intact) and pre-assign unique Sample IDs (see Section 11).

At the site — approach & setup

5. Conduct safety brief; position traffic cones if near road; avoid sampling during peak traffic unless safe.
6. Sanitize gloves and prepare sampling pole / rope / bucket. Do not remove bottle caps until ready to collect.

STEP-BY-STEP FIELD PROCEDURE



Collecting the sample

7. Remove cap without touching inside; hold bottle near the neck only.
8. If using a pole, bailer or rope, lower it into the flowing stream and collect from midstream or representative mid-depth (not surface scum). For manholes, lower via pole/bailer to sample mid-flow. Avoid splashing; do not touch the bottle rim to surfaces.
9. Fill bottle to the recommended fill volume for the lab (usually leaving 2–3 cm headspace for mixing). For microbiological/molecular tests, fill in completely per lab instructions.
10. Replace the cap tightly and seal with parafilm and wipe the exterior with disinfectant (alcohol wipe), then place in a secondary bag.
11. For multiple samples at the same site, change gloves between samples to avoid cross-contamination. Label each bottle immediately (do not wait until after moving to the next site).

On-site metadata & documentation

12. Complete COC and field log: sample ID, date/time, sampler name, site, sample type (grab), container volume, preservative used (if any), field observations (temperature, flow, odor, rain), and cooler ID. Record exact collection time to the minute.

Preservation & packing

13. Immediately place bottles upright into the cooler surrounded by pre-frozen ice packs so the bottle temp is ~2–8 °C. Do not freeze samples. Put a thermometer/data logger inside the cooler and note the starting temperature. Keep samples in a closed cooler and minimize opening.

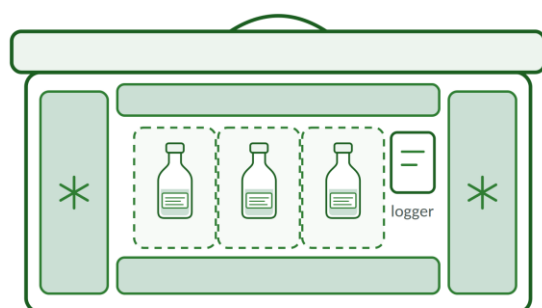


For the reference please view: https://drive.google.com/file/d/1f5XmMOVawyZYFmmtmAauP2_C9yqh-A9a/view?usp=drive_link

10 Cold-Chain Transport & Temperature Monitoring

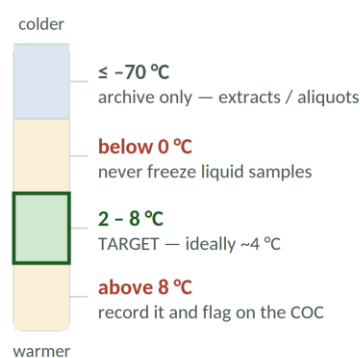
- **Target temp:** 2–8 °C (ideally ~4 °C). Use pre-frozen gel packs; place samples in plastic bags to avoid direct contact with melting ice. DO NOT freeze liquid samples (freezing can damage the integrity of RNA/microbes).
- **Transport container:** well-insulated cooler with a tight lid, thermometer, or electronic data logger to record temperature during transport. Seal cooler with tamper-evident tape if necessary.
- **Transit time:** aim to deliver to the lab same day; process within 24 hours if possible. If unavoidable, refrigeration up to 72 hours is acceptable, provided the lab adds matrix controls / documents hold time; for long-term archiving, store at ≤ -70 °C upon arrival.
- **On arrival at the lab:** receiving staff must record arrival time, cooler temperature, integrity of seals, and sign the COC. Lab should note if the sample temperature was > 8 °C or if the hold time exceeded recommendations.

A · HOW TO PACK THE COOLER



ice packs on all sides · bottles upright & bagged · logger inside · keep lid shut

B · TEMPERATURE ZONES



For the reference please view: https://drive.google.com/file/d/1f5XmMOVawyZYFmmtmAauP2_C9yqh-A9a/view?usp=drive_link



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11 Sample Labeling & Unique ID Format

Every bottle must have a waterproof label (printed or permanent marker) containing:

ID format (consistent & unique)

PK - <SITECODE> - <YYYYMMDD> - <HHMM> - <S#>

Example: PK-RWP_OPD001-20251016-0830-S1

Label fields (minimum)

- Sample ID (unique)
- Site name or code & GPS coordinates (optional)
- Date and time of collection (YYYY-MM-DD HH:MM)
- Sampler initials
- Sample type: Grab
- Requested analyses / preservatives (if used)
- Cooler ID and COC number

Also record metadata in the field log and COC form (see Section 12).



For the reference please view: https://drive.google.com/file/d/1f5XmMOVawyZYFmmtmAauP2_C9yqh-A9a/view?usp=drive_link

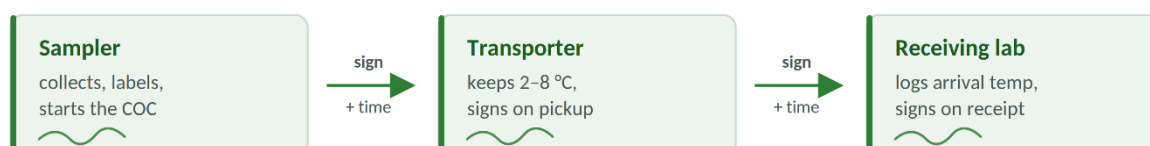
12 Chain-of-Custody (COC)

Each cooler / shipment must be accompanied by a COC that includes project/client, sample IDs, collection date/time, sampler name and signature, sample count, container types, requested analyses, preservatives, and signatures/time of relinquish and receive at each custody transfer. EPA or lab COC form templates can be used. Keep one copy with the shipment and retain copies in the project file.

COC quick fields (suggested)

Field	Entry
COC No.	
Project name & contact	
Cooler ID / Seal No.	
Sample ID(s) list	
Date / time collected	

CUSTODY TRANSFER — EVERY HANDOVER IS SIGNED AND TIMED



One COC copy travels with the cooler · one copy stays in the project file

Field	Entry
Collected by (name & signature)	
Date / time shipped	
Transporter (name & signature)	
Date / time received at lab	
Temperature on receipt (°C)	
Received by (signature)	

13 Holding Times & Processing Recommendations

Goal: process within **24 hours** refrigerated (2–8 °C). If the lab cannot process within 24 hours, refrigeration is acceptable up to 72 hours, but the lab must use matrix recovery controls and document that hold time. For archiving, freeze extracts or aliquots at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

14 Decontamination & Waste Disposal

- Disinfect exterior of bottles with 70% isopropanol before packing; disinfect reusable equipment and sampling poles between sites.
- Dispose of used gloves, wipes, and contaminated PPE in designated biohazard bags following local regulations; if unavailable, collect and incinerate or dispose of per municipal hazardous waste rules. Laboratory and field teams should follow local biosafety rules.

15 Record Keeping & Reporting

Maintain field logbook with photographs (site, sample IDs, cooler, flow) and GIS coordinates. Retain COC and field notes with project records for audit (EPA recommends retention). For surveillance reporting, follow the NWSS metadata fields (time, sample type, flow, site ID).

16 Special Considerations for Pakistan

- **Climate and heat:** summer ambient temperatures can be high; minimize time outside, and pre-freeze extra ice packs for the cooler. If sampling is done in hot weather, plan morning sampling and express transport to the lab.
- **Access & security:** open drains may be in busy or unsafe areas; proper coordination should be done with local authorities. Manhole access may require utility permission and confined-space precautions.

APPENDICES

Appendix A — Representative Site Selection Table (Examples)

Site code	Type	Where to sample (practical)	Why / notes
LAH_OPD001	Open drain (residential)	Midstream, ~5–10 m downstream of bend, use pole to reach mid-flow	Avoid stagnant pockets; record nearby drainage inflows
KAR_MH005	Closed drain (manhole)	On channel floor where flow concentrates (mid-depth) using bailer	Use confined space precautions if entering
ISB_PS02	Pumping station (influent)	Wet well mid-depth or influent channel upstream of pumps	Sample before chemical dosing or mechanical agitation
PES_OUT01	Outfall to river	Immediately before discharge point (midstream if possible)	Document river ingress/flow and tidal effects

Modify codes to your local site naming convention and add GPS coordinates.

Appendix B — Example Sample Label (Printable)

WES — WASTEWATER GRAB SAMPLE	
Sample ID	PK-<SITE>-YYYYMMDD-HHMM-S#
Site	<site name>
GPS	<lat, long>
Date / Time	YYYY-MM-DD HH:MM
Sampler	<initials>
Type	Grab
Cooler ID	<ID>
COC No.	<#>

Appendix C — Quick Field Checklist

- ☐ Cooler prepped with ≥ 2 ice packs?
- ☐ Sterile bottles intact & labeled?
- ☐ Gloves, pole, disinfectant, COC form present?
- ☐ Field log & GPS/phone camera ready?
- ☐ Cooler thermometer / data logger?

- ☐ Transport arranged to the lab within the target time?

Appendix D — Grab Sampling Video

Demonstration video prepared by the WES, NIH team:

[Watch the grab sampling demonstration →](#)

https://drive.google.com/file/d/1f5XmMOVawzyZFmmtmAauP2_C9yqh-A9a/view?usp=drive_link

Contact Us

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References

Key authoritative sources used to prepare this document.

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2. **CDC — Developing a Wastewater Surveillance Sampling Strategy & NWSS.** Sample type, metadata to record, storage recommendations — process within 24 h if possible; refrigerate at 4 °C. archive.cdc.gov/www_cdc_gov/nwss/sampling.html
3. **APHL — SARS-CoV-2 Wastewater Surveillance Testing Guide.** Laboratory handling, storage and processing recommendations; refrigerated storage up to 4 days if needed. [EH-2022-SARSCoV2-Wastewater-Surveillance-Testing-Guide.pdf](#)
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6. **Pakistan — Ministry of Climate Change (NEQS) & PCRWR.** National environmental standards and local water quality agencies, for regulatory alignment. mocc.gov.pk
7. **U.S. EPA — Chain-of-custody form & guidance.** COC best practice. [sample-chain-custody-form.pdf](#)