geno-MIC

microbiologically influenced corrosion

# STANDARD OPERATIONG PROCEDURES

**for**

# SAMPLING ONSHORE ASSETS

**for**

# GENOMIC and MICROBIAL EXPERIMENTS

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Version 1.2 – September 2018

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# Important Notes

1. Avoid contamination of samples.
    a. Wear lab/latex gloves when collecting samples.
    b. Do not touch the inside of containers and lids
    c. Do not reuse needles and syringes and sampling tools (spatulas, scrappers)

2. Some sample containers contain DNA preservative. Do not discard this preservative.

3. Samples should be place in a cooler with ice/ice packs immediately.
    a. Do not freeze samples.
    b. Do not expose samples to extreme heat or sun light.
    c. Ship samples as soon as possible and with enough ice to last at least four days in transit.

# Summary of Samples Requested

| Sample Type | Source | Sample Container | Sample Amount | Number of samples | Preservative in container | Analysis to be performed |
|---|---|---|---|---|---|---|
| liquids | source or produced waters | 1 L plastic bottles | 1 L | 1 | yes | genomic (DNA) analysis |
| | | 1 L plastic bottles | 1 L | 3 | no | microbial assays |
| | | 100 ml glass serum bottles | 50 ml | 2 | no (beads or coupon) | On-site corrosion tests |
| sludge, solids | pigging material, internal swabs, scraps | 50 ml tube | 5 – 20 g | 2 | yes | genomic (DNA) analysis |
| | | 250 ml jar | 200 g | 1 | No | microbial assays &/ corrosion tests |

# Liquids Sampling

**This sampling protocol is to be used for collecting liquids (e.g., produced waters either from flowing or static systems) from onshore oil and gas operations.**

**These samples will be used for:**

1. **Genomic analysis (sampling vessels will contain a preservative)**
2. **Live microbiological analysis**
3. **On-site initiation of corrosion tests**
4. **Bench-top MIC testing (if enough field water sample is provided)**

## Supplies for liquids sampling (Figure 1)

- Four sterile 1-L wide mouth plastic bottles for collecting bulk water samples
    - 1 bottle will already contain preservative for genomic analysis (DO NOT DISCARD THIS LIQUID) – **THIS WILL BE LABELLED 'PRESERVATIVE (P)'**
    - 3 bottles will contain no preservative
- 2 glass serum bottles that are stoppered, each containing a corrosion coupon or beads
- 50 mL Syringe and needle (sterile, wrapped separately)
- Latex or nitrile gloves
- Labeling tape and Sharpies
- Cooler and ice packs
- Make sure you add bubble-wrap bags for the glass serum bottles

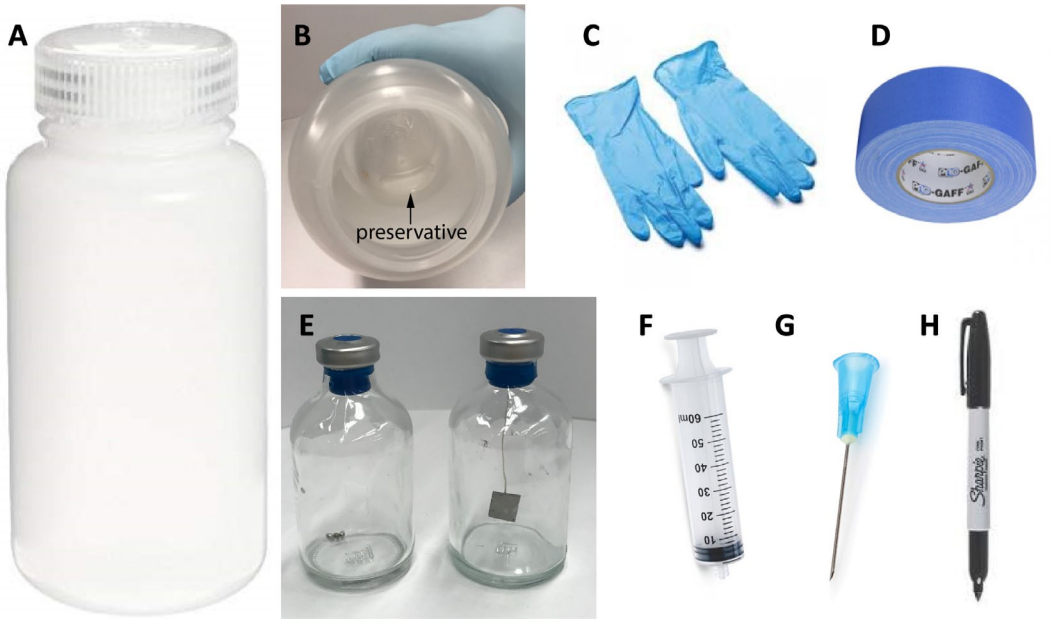


Figure 1: Supplies for liquids sampling. (A) 1-L Nalgene bottle, (B) 4ml preservative already added to the Nalgene bottle and should not be discarded, (C) latex/nitrile gloves, (D) labelling tape, (E) glass serum

bottles for onsite corrosion tests, containing three low carbon steel beads or corrosion coupon, (F) syringe, (G) needle, (H) permanent marker.

## Liquids Sampling using 1L bottles (Figure 2)

1. Put on latex/nitrile gloves, use a clean pair for each sampling location. Label each bottle with the sampling location, date (and other information if known, as outlined on the attached sample check-list).
2. Sample may be collected from either flowing stream (e.g. pipeline) or static (e.g. storage tank) systems.
    a. From flowing process liquid - samples shall be collected by slowly opening the sample point and adjusting the flow to a steady rate.
    b. From dead space fluid – sample shall be collected with precaution not to introduced other variables, such as oxygen, and avoid turbulent flow while sampling/opening valves.
    c. The fluid should be allowed to flow to thoroughly flush out dead-space fluids and solids before the samples are collected. It is recommended to purge the water line with at least a couple of volumes of liquid (e.g., minimum of 1-L) before collecting sample.
    d. During sampling of systems containing both oil and water, phase separation should be permitted to occur before the water is used. However, it is satisfactory to directly use an emulsion for bacterial isolation.
3. Unscrew the sample bottle, taking care not to touch the brim of the sample bottle or the inside of the lid; set lid down in an inverted manner on a flat surface.
4. Fill each bottle to the brim (so that it contains no air), and close the lid, taking care not to touch the inside of the lid.

    **Note:** For the 1-L bottle containing the preservative – do not overflow the bottle as the preservative will be lost.
5. Invert the sampling bottle a few times to mix.
6. Label bottle (if not already labeled) with the sample ID, and date. Keep one bottle out - not **the one with preservative** – pick a different bottle in order to subsample to initiate corrosion experiments, see protocol below entitled 'On-site corrosion initiation experiments'

    **Note:** Oil can cause tape to come off and Sharpie writing to blur, so be sure and write ID information in two different locations, such as the side of the bottle and the lid.

    **Note:** Keep a more detailed record of sample information in a notebook and/or Excel spreadsheet or data collection sheet.
7. Place remaining samples in a cooler (Figure 2).
8. Take a digital photograph of the sample container (after filling) and your field notes.

9. Proceed to initiate on-site experiments as described below.

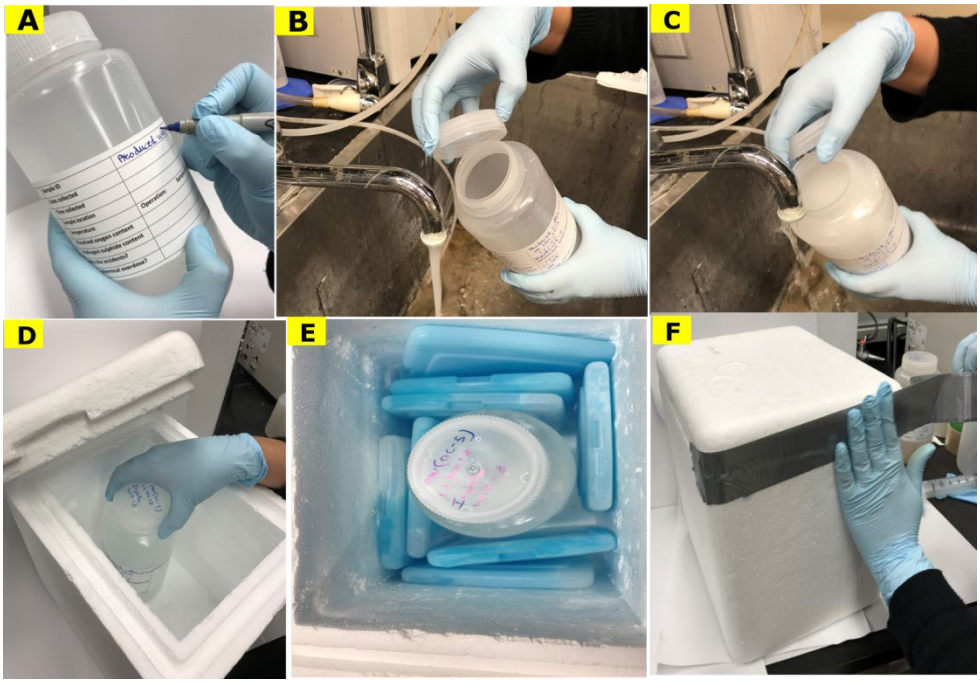


Figure 2: Representation of steps to be followed while collecting liquid samples. (A) Wear gloves and label the 1-L Nalgene bottles, (B) Allow the fluid to flow after opening the fluid source to thoroughly flush out dead-space fluids, (C) Fill the Nalgene bottle till the mouth to avoid air, (D) Label the lid of the bottle and transfer it to insulated container, (E) Place the ice packs around the sampled bottles, (F) Seal the box on all sides, stick a sheet with the box details on top /side of the box and send it for shipment.

**Note:** make sure you take samples for onsite corrosion testing (corrosion serum bottles) before shipment

## **On-site corrosion initiation experiments** (Figure 3)

1. Put on clean latex/nitrile gloves.
2. Prepare the syringe and connect to the needle from their sterile packings.
3. Open the 1-L sample bottle kept out of the cooler (again, placing lid down in an inverted manner) and remove 50 mL using the syringe and needle.
4. Dispense this sample into the glass serum bottles containing test metal inside (beads/coupon). Insert the needle through the stopper, then push on the syringe piston to add liquid into the bottle. This will become difficult near the end as there will be backpressure. Once the liquid is added, let go of the syringe piston in order to release the backpressure (will be about 50 mL) and remove the syringe from the stopper.
5. Repeat for the second bottle.
6. Label bottles with the sample ID, date, and sample volume, place in in bubble-wrap bags and add to the cooler.
7. Close lid of 1-L bottle and add to the cooler.
8. Tape up cooler for shipping.

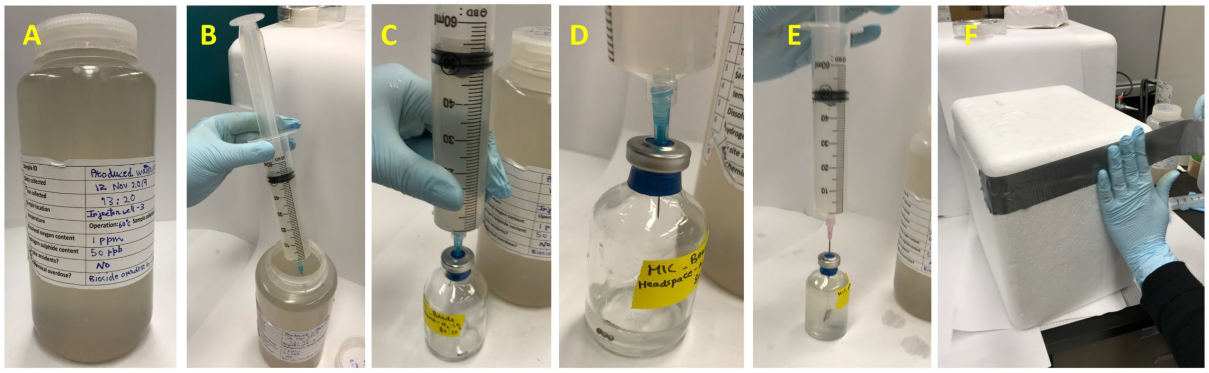


Figure 3: Representation of steps to be followed while collecting liquid samples for initiating on-site corrosion tests. (A) Wear gloves and use a sample already collected (not the one containing a preservative), (B) Using a syringe attached with a needle, collect 50 mL fluid sample from this test bottle, (C) Inject this fluid into MIC test bottle containing beads/coupon, (D) fill the MIC test bottle with 50 ml volume by gently releasing the contents of the syringe into the bottle, (E) release the back pressure of the sampling bottle to 50 mL, (F) Label the bottle and transfer it to insulated container and seal the box on all sides, stick a sheet with the box details on top/side of the box and send it for shipment.

# Solids Sampling from Pigging Operations

**This sampling protocol is to be used for collecting solids (sludge, debris) from pig receivers (pig traps) following a pigging operation.**

**These samples will be used for:**

5. **Genomic analysis (sampling vessels will contain a preservative)**
6. **Live microbiological analysis and microbiologically influenced UDC (MI-UDC) testing**

## Supplies for solids sampling from pigging operations (Figure 4)

- Latex or nitrile gloves
- 2 sterile plastic conical tubes pre-loaded with a preservative (will be labeled 'Genomic Analysis')
- Sterile synthetic swabs (2 per package; for Genomic analysis)
- Sterile 250-mL wide-mouth plastic jar (will be labelled 'Microbiological Analysis')
- Sterile sampling spoons (for Microbiological analysis)
- Ziploc bags (1-L)
- Permanent marker for labeling tubes and jars
- Cooler and ice packs

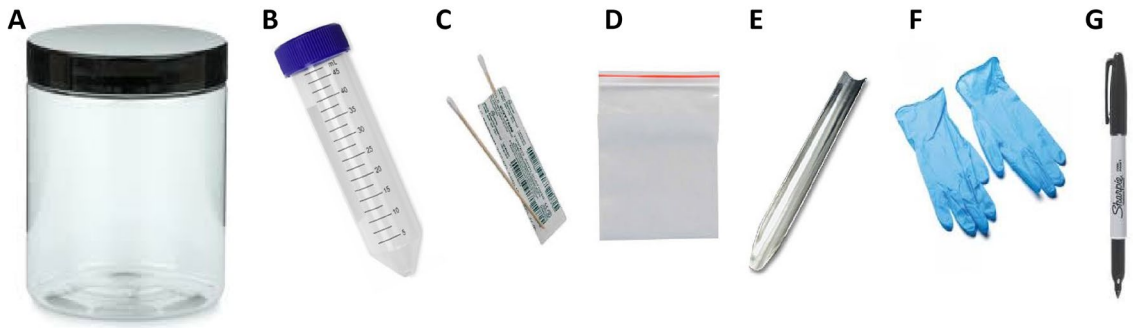


Figure 4: Supplies for collecting pig receiver samples. (A) sterile 250 mL wide-mouth plastic jar, (B) 50 mL centrifuge tube pre-loaded with preservative – DO NOT DISCARD THIS LIQUID, (C) cotton swabs, (D) (E) sampling spoon, (F) latex/nitrile gloves, (G) permanent marker.

## Protocol for sampling pig receiver (trap) samples:

### Solids sampling using 50-mL conical tubes for Genomic Analysis (Figure 5)

1. Put on a clean pair of latex/nitrile gloves.
2. Prepare one 50-mL tube (labeled 'Genomic Analysis') to receive a sample by opening the lid, and placing it down on a surface (could be on the ground if needed) in an inverted manner. Take care not to touch the inside of the lid. DO NOT DISCARD THE LIQUID – THIS IS THE PRESERVATIVE.
3. Tear open the swab package near the top (away from the swab end).
4. Remove one sterile swab, holding within the top inch of the wooden shaft, and use the swab to collect a large a glob of material as possible.
5. Insert the swab into the conical tube, and swish around to make sure the preservative contacts the sample material.
6. Break off excess wooden portion of the cotton swab so that it fits into the conical tube when closed—try to break at a point lower than your fingers have touched. Close the lid tightly.
7. Repeat the same protocol (steps 2-6) with the second 50-mL tube and the other swab from the package
8. Clearly label the tubes with sampling location and date.
9. Take a digital photograph (if phone/camera permitted at the site) of the sample container (after filling) along with your field notes.

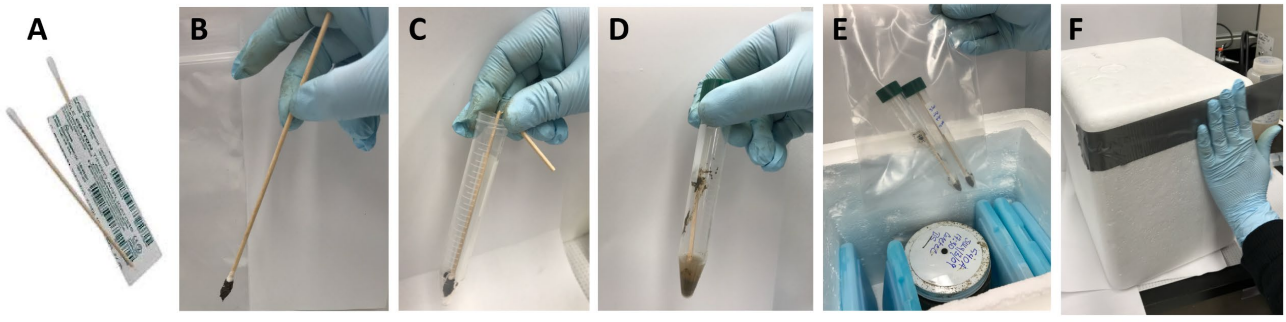


Figure 5: Representation of steps to be followed while collecting pig solids samples for genomic analysis. (A) Take one sterile cotton swab from the package, (B) collect a big chunk of sample using the swab, (C) insert sample into the liquid in the conical tube and break off the excess wooden portion of the swab so that it fits into the conical tube when closed (D), (E) Label the tube and take one more sample into a second tube to get replicate samples and seal both the samples in the Ziploc bag, (E) Transfer this Ziploc bag to insulated container and place the ice packs next to the sample bag, (F) Seal the box on all sides, stick a sheet with the box details on top /side of the box and send it for shipment.

## Solids sampling using 250-mL plastic jars for Microbiological Analysis and MI-UDC Testing (Figure 6)

1. Wearing latex/nitrile gloves, loosen lid of the sterile sampling bottle and place the lid down on a surface (could be on the ground if needed) in an inverted manner.
2. Tear open the large sterile sampling spoon package near the top (away from the sample holder).
3. Remove one sterile sampling spoon, and use it to take a sample from the pig receiver (the sample shall be representative of the fresh solids collected) - collecting as large a portion of material – ideally to near the top of the sampling bottle (**we are looking for about a 'regular coffee cup-sized' sample**). Close the cap tightly.
4. Discard dirty spoon into a Ziploc bag.
5. Clearly label the bottle on the side and lid with sampling location, pig number (i.e. 1, 2 etc.), and date.
6. Take a digital photograph of the sample container (after filling) along with your field notes.

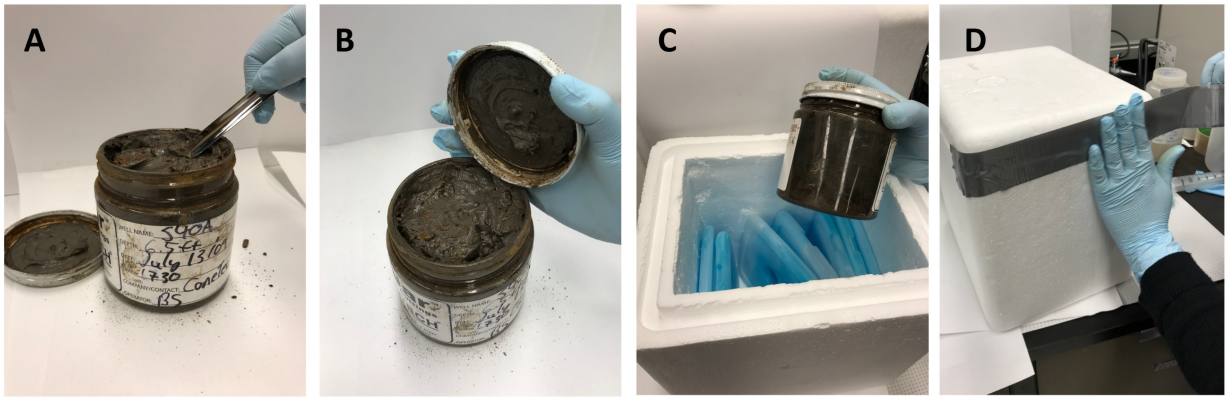


Figure 6: Representation of steps to be followed while collecting solid pig samples for live microbiological analysis. (A) Fill the sampling bottle with solid sample using a sterile sampling spoon. (B) Completely fill the sampling bottle till the mouth of the container and label it on the side and on the cap of the container. (C) Transfer this bottle to insulated container and place the ice packs, (D) Seal the box on all sides, stick a sheet with the box details on top /side of the box and send it for shipment.

# Solids Sampling from Internal Pipeline Surfaces

**This sampling protocol is to be used for collecting solids from pipeline surfaces (which may also include corrosion coupon surfaces)**

**These samples will be used for:**

7. Genomic analysis (sampling vessels will contain a preservative)
8. Live microbiological analysis and microbiologically influenced UDC (MI-UDC) testing

*Given that pipeline solids may not always be plentiful, it is acknowledged there may not be enough sample for this analysis*

## Supplies for solids sampling from internal pipeline surfaces (Figure 7)

- Latex or nitrile gloves
- 6 sterile 50 mL tubes pre-loaded with a preservative (labeled 'Genomic Analysis')
- 3 packages of sterile synthetic swabs (2 per package; for Genomic analysis)
- 3 sterile 50-mL conical tubes, no preservative (labelled 'Microbiological Analysis')
- 3 sterile sampling spoons or spatulas (for Microbiological analysis)
- Ziploc bags (1-L)
- Sharpies for labeling tubes and bottles
- Electric tape or parafilm to seal the tubes after sampling
- Cooler and ice packs

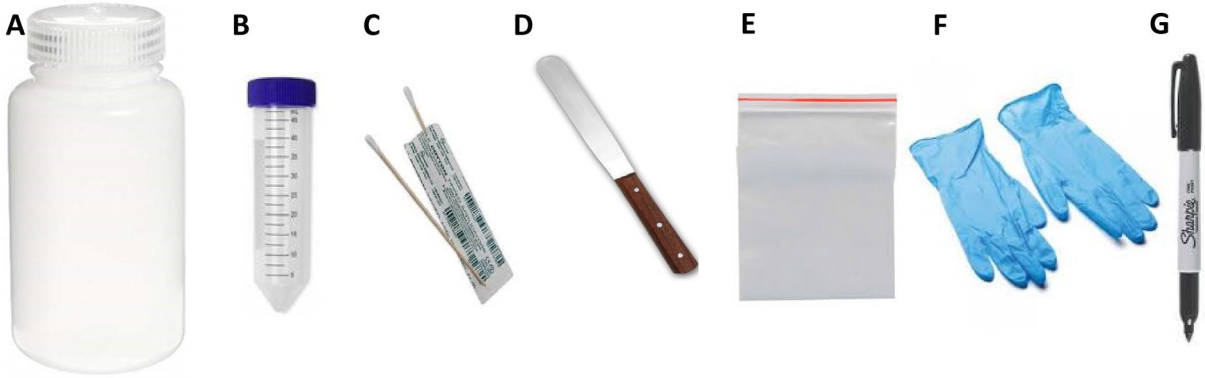


Figure 7: Supplies for collecting pipeline solids were MIC is suspected. (A) sampling bottle (if there is associated fluids available for testing) (B) 50 mL conical tubes – 6 containing preservative, P; 6 not containing preservative, NP; (C) sterile cotton swabs (may use synthetic swabs instead, (D) sterile sampling knife or spoons, (E) Ziploc bags, (F) latex/nitrile gloves, (G) permanent marker.

**Ideal locations for solid samples collected from internal surfaces** (Figure 8)

1. *Leak Site:* The location of the leak (where MIC may have occurred). Please indicate whether the samples were obtained from inside, outside or distant from the pits. It is preferred that samples are collected from the pits as the location where the leak occurred may have washed away the solids samples or has caused cross contamination from the surrounding area.
2. *Adjacent Site:* A location adjacent to the leak (because leak testing may have washed away or introduced microbes at original leak site, but a site adjacent to the leak will still have a chance of containing MIC microbes)
3. *Control Site:* A 'control' location away from the pitting area or where there is no pitting (uncorroded control)

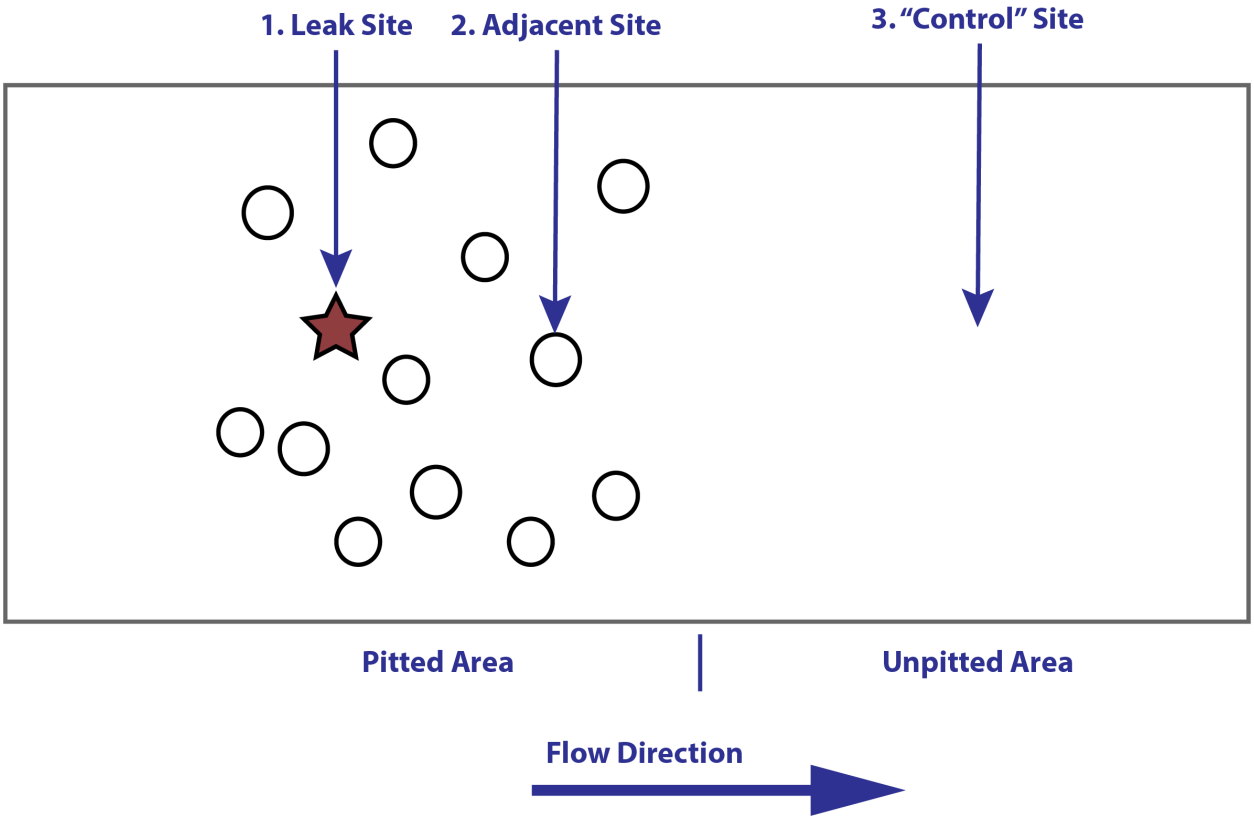


Figure 8: Internal sampling locations around a leak.

## Protocol for sampling internal pipeline solids

do for EACH of the three pipeline sites indicated above:

1. Put on latex/nitrile gloves. Use a clean pair for each sampling site.
2. For each site, it is ideal to first take 2 swab samples for genomic analysis, and also to collect solids for live microbiological analysis if enough sample is present. Take samples for genomic analysis first, then collect any additional solids for live microbiological tests:

Note- make sure you take pictures before sampling and indicate the location on the pictures where the samples were taken, and indicate the orientation of the pipe (i.e. O'clock positions) and the direction of flow.

### Sampling Interior of Pipelines for Genomics Analysis **(Figure 8)**

1. Loosen the lid of one conical tube labeled 'Genomics Analysis'. Set the lid down on a surface in an inverted manner, taking care not to touch the inside of the lid.
2. Tear open the swab package near the top (away from the swab end).
3. Remove one sterile swab, ensuring to hold the swab near the top 2.5 – 3 cm. Use to swab the inside of the pipe, going back and forth and upwards and downwards in an area of about 6 $cm^2$ (~ 2.5 cm X 2.5 cm).

   **NOTE:** if the pipe material appears dry – dip the swab into the conical tube containing the liquid preservative first to moisten, then use to swab.
4. Insert the swab into the conical tube, and swish around to make sure the preservative contacts the sample material.
5. Close the tube with the lid, ensuring that the lid is seated/sealed well.
6. Using a second conical tube containing preservative and the second swab from the package, repeat steps 3-7. You should have 2 swab samples from each location for Genomic Analysis.
7. Clearly label the tubes with sampling location and date.
8. Make sure the lids are taped or parafilm; and add both tubes to a Ziploc bag, and place into a cooler containing ice packs.
9. Take a digital photograph of the sample container (after filling) along with your field notes.

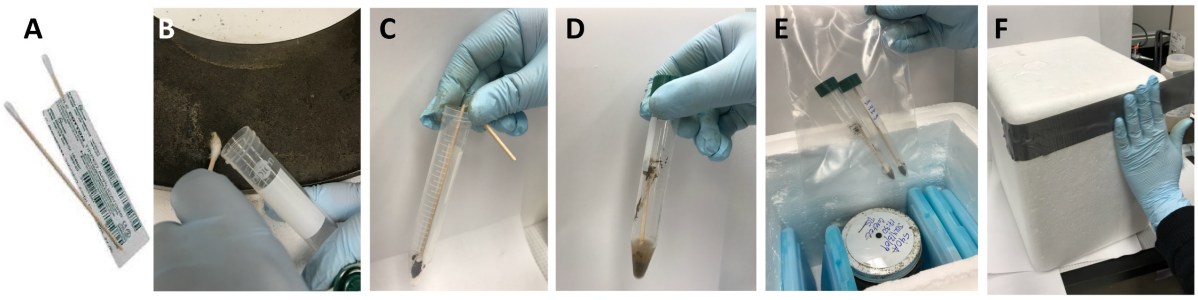


Figure 8: Representation of steps to be followed while collecting pipe solids samples for genomic analysis. (A) Take one sterile cotton swab from the package, (B) Collect sample from the surface of the pipe using the sterile cotton swab (moisten if needed), (C) Break off excess wooden portion of the cotton swab so that it fits into the conical tube when closed, (D) Label the tube and take one more sample to get a replicate and seal both the samples in the Ziploc bag, (E) Transfer this Ziploc bag to an insulated container and place ice packs next to the sample bag,

## Sampling Interior of Pipelines for Microbiological Analysis and MI-UDC Testing (Figure 10)

Collect the following additional set of samples if there are additional solids on the pipe

1. Loosen the lid of one conical tube labeled 'Microbiological Analysis'. Set the lid down on a surface in an inverted manner, taking care not to touch the inside of the lid.
2. Remove one sterile sampling spoon, and use it to take sample from the surface, adding scrapings into the sterile tube. Seal the tube, ensuring the lid is well seated/sealed.
3. Repeat for a second tube (if possible).
4. Make sure the cap is well seated/sealed when finished.
5. Clearly label the tube with sampling location and date.
6. Add both tube(s) to a Ziploc bag, and place into a cooler containing ice packs.
7. Take a digital photograph of the sample container (after filling) along with your field notes.


Figure 9: Representation of steps to be followed for collecting solid samples for Microbiological Analysis and MI-UDC Testing. (A) Use the sterile sampling spoon provided in the kit as shown in the picture, (B) Scrape the surface of the pipe using the spoon, and collect as much sample as possible into the sterile tube; label tube on the side and on the cap of the container, (C) Using a separate sterile knife repeat step (B) for any additional sludge adhered to the pipe's surface, (D) Transfer spoons used for sample collection into Ziploc bags and label the bag, (E) Transfer the Ziploc bags containing the knife and the tubes into an insulated container and place the ice packs, (F) Seal the box on all sides, stick a sheet with the box details on top /side of the box and send it for shipment

# Sample Shipment

1. After collection, samples should be stored in the dark.
2. Place ice packs around sample containers to bring temperatures down to within the range of 1 to 4$^{o}$C.
3. Prolonged storage of longer than a few days should be avoided.

   **Note:** Store all samples on ice once taken – **keep cold but do not freeze** (this can ruin the integrity of DNA).
4. Ship the samples to the lab in a cooler with ice or ice packs.

## Shipping Addresses

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