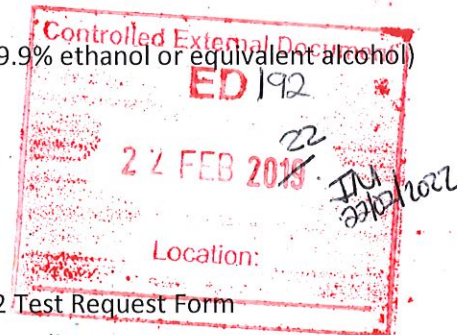


**Title: Sampling of water, swabs and air quality settle plates, sample handling
and transportation procedures****1. Purpose and Scope**

This document outlines the procedures to be followed during water, swab and air quality settle plate sampling as well sample conditions to be met upon sample submission, sample handling and transportation of samples to the laboratory.

2. Equipment and consumables

- 2.1. Sterile water sampling bottles 250ml or 500ml or 1000mL propylene (autoclavable)/ sterile disposable sampling bottles.
- 2.2. Sterile sampling cup (sterile both inside and out and protected, for example, by Kraft paper to keep dry after autoclaving, aluminum foil or by plastic outer bags)
- 2.3. Sterile disposable water sampling bottles containing sodium thiosulphate
- 2.4. PCA/CGA Agar plates wrapped with parafilm (for Air Quality Settle Plates sampling)
- 2.5. Nitrile Powder free gloves
- 2.6. Cooler box
- 2.7. Sampling bag (Applicable when sampling is taking place on vessels)
- 2.8. Hard Ice bricks or soft ice gels
- 2.9. Lighter
- 2.10. Blow torch
- 2.11. 70% Alcohol and Absolute alcohol (99.6%/99.9% ethanol or equivalent alcohol)
- 2.12. Cotton wool
- 2.13. Beaker
- 2.14. Permanent marker pen
- 2.15. Sterile swab sticks with transport fluid
- 2.16. Sterile forceps or Sterile sampling pole
- 2.17. Document: Microbiology Section F QM 7.1-2 Test Request Form
- 2.18. Purchase order from customer requesting sampling

**3. Safety and personal protective equipment**

- 3.1. The basic personal protective equipment (PPE) when working with this procedure is a laboratory coat, Safety shoes, gloves, Safety helmet (applicable on vessels) and a reflector vest (applicable on vessels). Precautions should be taken to avoid inhalation of toxic gases and ingestion of toxic materials through the nose, mouth and skin when using the blow torch.

Procedure for sampling of water (from different sources) and ice samples [based on ED 294, 411 & 412 and ISO 19458] and sampling of swab samples (based on ED213, ED 420)

4. Collecting water samples from taps

4.1. Procedure

- 4.1.1. Collect sterile bottles from the laboratory for sampling (note: an exception is given to bottled water where the bottle is deemed suitable for acceptance by the laboratory).
- 4.1.2. A container with minimum capacity of 250 ml, 500ml or 1000ml should be used to collect water samples. The container capacity is selected based on the number and type of tests requested.
- 4.1.3. Mark the bottles using a permanent marker pen with sample identification, date and time of sampling.
- 4.1.4. Open the tap and let it run/flush for at least 3 minutes to allow the water that was already in the pipe to run out. Do not sample from taps with leaking spindles and avoid mixer taps, if possible.
- 4.1.5. In the case any faucet nozzle or other attachment or insert is present remove it (spanners and pliers shall be available). Scrape off any dirty (scale, slime, grease or other extraneous matter) and fully open and close the tap repeatedly to rinse out (flush) the dirt from the tap.
- 4.1.6. Close the tap and disinfect.
- 4.1.7. Disinfect metal taps by heating with a blow torch on the sides and at the tip, (note after flaming and opening the tap a sizzling noise should occur).
- 4.1.8. Disinfect plastic tap by wiping the outside of the tap with absolute ethanol (99.9%)/ isopropanol or any other equivalent disinfectant and the inside if possible, using cotton wool or a cotton swab soaked in alcohol by dipping it in the tip of the tap for a short while after thorough cleaning.
- 4.1.9. Open the tap and let the water run slowly for a short while, to ensure that the sample has no residual thermal or disinfectant effect.
- 4.1.10. With powder free nitrile gloves on (disinfect gloves with alcohol), remove the cap of the sampling bottle, taking care not to touch and contaminate the inner surface of the cap or neck of the bottle with hands, and place the open bottle in the flowing water to collect sample, without closing and reopening of the tap.
- 4.1.11. Fill the bottle up to the neck leaving airspace (at least 3cm) to allow for shaking during analysis and replace the bottle cap immediately.
- 4.1.12. Close the tap.
- 4.1.13. Take note, to disinfect hands/ gloves using 70% or 99.6% or 99.9% ethanol or equivalent alcohol between collection of different water samples.
- 4.1.14. When collecting multiple water samples from the same source with the desire to attain repeatable results, after collecting the first sample, repeat the same procedure from 4.1.10 to 4.1.12.

Note:

- **Sampling from drinking water storage tanks is usually made from a tap on the outlet.**
- **Subsurface samples taken from the tank itself, in which case, use bottles that are sterile both inside and outside. Here the entire bottle is wrapped in Kraft paper. (Alternatively, the outside of the bottles may be disinfected prior to sampling by using a suitable disinfectant such as isopropanol/ 99.9% ethanol and is allowed to dry before use).**
- **Disinfect hands/gloves using the 70% to 99.9% ethanol/ isopropanol prior to sampling and between different water samples collected.**

- 4.1.15. Place the sample into the cooler box/ sampling bag with the ice bricks for transportation to the laboratory (transport wastewater samples separately from drinking water samples).

4.1.16. Customer to ensure completeness of Test Request Form: Microbiology Section F QM 7.1-2 during the sampling process by recording the sample identification name, date and time of sampling for each water collected, and test request form to accompany samples to the laboratory during submission.

4.1.17. Cool samples during transport (e.g. by using ice packs or melting ice) Take care not to freeze them. Protect samples from sunlight.

5. Collecting of subsurface water samples from rivers, streams, lakes, dams, seaside, swimming pools and waste waters

4.1.18. 5.1. Procedure

4.1.19. Collect sterile bottles (from the inside and outside) from the laboratory for sampling.

4.1.20. Mark the sterile bottles using a permanent marker pen with sample identification, date and time of collection.

4.1.21. With gloves on remove the cap of the sample bottle and collect the water using the sterile sampling bottle from inside and outside, taking care not to touch and contaminate the inner and outer surface of the cup with your hands.

4.1.22. Bathing waters (rivers, lake and seaside) in a 1 m to 1.5 m water column:

4.1.22.1. Submerge the sterile bottle with neck downwards below the water surface to a sampling depth of (-20cm to -30cm) using stainless steel sterile forceps.

4.1.22.2. Fill the bottle by turning it sideways and upwards to prevent contamination of the sample, where current exist hold the bottle upstream

4.1.22.3. Where the water column at beaches is less than 1 m, care should be taken not sample to close to the bottom to reduce variation and possible resuspension of bacteria.

4.1.23. Swimming pool

4.1.23.1. Submerge the sterile bottle horizontally to avoid losing of thiosulphate below the water surface to a sampling depth of (-10cm to -30cm).

4.1.23.2. Then turn the bottle upright until enough water is collected

4.1.24. Waste waters

4.1.24.1. In addition to using disposable and powder free gloves a sterile sampling pole or forceps may be used to reduce infection of sampler.

4.1.24.2. Submerge bottle horizontally and scoop sample away from the direction of the hand to collect the sample

4.1.24.3. Remove the dirt from the outer surface of the bottle and place in clean plastic bags.

4.1.25. Ensure sufficient airspace by filling bottle up to the neck to allow for shaking during analysis and replace the cap immediately.

Note: Disinfect hands/gloves using the 70% to 99.9% ethanol/ isopropanol prior to sampling and between different water samples collected

- 4.1.26. Place the sample into the cooler box/ sampling bag with the ice bricks for transportation to the laboratory (transport wastewater separately from drinking water).
- 4.1.27. Ensure completeness of Test Request Form: Microbiology Section F QM 7.1-2 during sampling process by recording the sample identification, date and time of sampling for each water sample collected, and test request form should accompany samples during submission to the laboratory.
- 4.1.28. Cool samples during transport (e.g. by using ice packs or melting ice). Take care not to freeze samples. Protect samples from sunlight.

Note: Warm and cooler samples shall be transported separately. Keep the time between sampling and analysis in the laboratory as short as possible. For drinking waters, analysis should ideally be started within the same working day.

6. Collecting ice samples

6.1. Procedure

4.2. Collecting ice samples

- 4.2.1. Collect sterile bottles from the laboratory for sampling.
- 4.2.2. A container with minimum capacity of 1000 ml should be used to collect ice samples or use 2x 500ml bottles to collect enough sample material.
- 4.2.3. Mark the bottles using a permanent marker with sample identification, date and time of collection.
- 4.2.4. With powder free nitrile gloves on (sterilise gloves with alcohol), remove the cap of the sample bottle, taking care not to touch and contaminate the inner surface of the cap or neck of the bottle with your hands.
- 4.2.5. Open the lid or remove cover from the ice container and fill the bottle up to the neck with ice by scooping forward away from the hands.
- 4.2.6. Immediately replace the cap of the bottle.

Note: Disinfect hands/gloves using the 70% to 99.9% ethanol/ isopropanol prior to sampling and between different water samples collected

- 4.2.7. Place the samples into the cooler box with the ice bricks for transportation to the laboratory.
- 4.2.8. Ensure completeness of Test Request Form: Microbiology Section F QM 7.1-2 during sampling process by recording the sample identification, date and time of sampling for each water sample collected, and test request form should accompany samples during submission to the laboratory.

7. Sampling of Air quality settle plates:

7.1. Procedure

7.1.1. Monitoring Total Viable Colony count (TVC)

- 7.1.1.1. Upon collection of sterile plates, customer should be in possession of a cooler box with ice bricks to maintain appropriate transportation conditions outlined in table 2 of this document.
- 7.1.1.2. **Analyst should wear powder free gloves and disinfect when performing this procedure.**
- 7.1.1.3. Acclimatise 2 plates of PCA to room temperature for each area to be monitored.
- 7.1.1.4. Mark the plates with the ID, date and type of the media.
- 7.1.1.5. Set timer for 15 minutes and expose/open both plates for 15 minutes on the same surface area.
- 7.1.1.6. The plates set in the biohazard hood should be exposed for 30 minutes.
- 7.1.1.7. Close the plates immediately, invert plates and seal off with parafilm provided by the laboratory. Place in plastic packaging and close before placing into the cooler box with ice bricks for transportation to the lab.

7.1.2. Monitoring yeast and Mould contamination

- 7.1.2.1. Upon collection of sterile plates, customer should be in possession of a cooler box with ice bricks to maintain appropriate transportation conditions outlined in table 2 of this document.
- 7.1.2.2. **Analyst should wear powder free gloves and disinfect when performing this procedure.**
- 7.1.2.3. Acclimatize 2 plates of Chloramphenicol agar to room temperature for each area to be monitored.
- 7.1.2.4. Mark the plates with the ID, date and type of the media.
- 7.1.2.5. Set timer for 15 minutes and expose/open both plates for 15 minutes on the same surface area.
- 7.1.2.6. The plates set in the biohazard hood should be exposed for 30 minutes.
- 7.1.2.7. Close the plates immediately, invert plates and seal off with parafilm provided by the laboratory. Place in plastic packaging and close before placing into the cooler box with ice bricks for transportation to the lab.

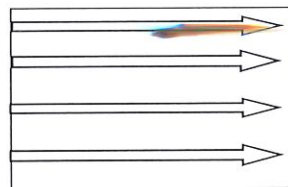
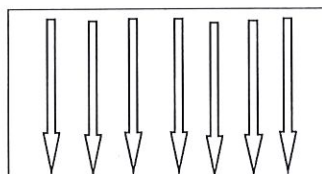
- NB:**
- **Temperature to be maintained during transport is 0 - 10°C.**
 - **Mode of transport is in a cooler box, on ice bricks.**
 - **Packaging should be in a closed packaging that will prevent contamination.**
 - **Sample label should be clear & legible, and correspond with what appears on the test request form.**
 - **Submission time tolerance from plate collection is recommended same day return, however up to 3 days is allowed provided that plates are kept refrigerated between 2 – 8 degrees before sampling.**

- **Plates shall be sealed with parafilm, and must shall be transported inverted.**

8. Collection of Swabs

8.1. Procedure

- 8.1.1. The laboratory provides sterile swabs in the event the customer does not have sterile swabs.
- 8.1.2. **Analyst should wear powder free gloves and disinfect hands/gloves using the 70% or 99.6%/99.9% ethanol between different swab samples.**
- 8.1.3. Peel the swab pack to expose the caps, dip the swab's tip into sterile transport fluid in order to pre-moisture the swab tip for maximum sample collection.
- 8.1.4. Prepare the sampling swab by marking with clear identification to swabbing area.
- 8.1.5. After surface cleaning and disinfection, carefully remove the pre-moistened swab and thoroughly swab by about 10cm x 10 cm = 10²cm by the pattern below:



Note: Important swabbing technique:

- **Do not touch swab or inside of sample device with fingers.**
- **Vigorously rotate swab while collecting sample to maximize sample collection on swab bud.**
- **Supply sufficient pressure to create flex in swab shaft.**
- **Swab in crisscross pattern vertically and horizontally directions.**

- 8.1.6. After swabbing immediately place swab into sterile transport fluid, avoid touching the walls of tube when inserting swabs.
- 8.1.7. Ensure swab is firmly pushed into the transport container to ensure no contamination can occur.
- 8.1.8. Ensure completeness of the test request form: Microbiology Section Form FQM 7.1-2 during the sampling process by recording the sample identification name, date and time of sampling for each swab sample collected, and the test request form should accompany the samples to the laboratory during submission.
- 8.1.9. For transportation of samples to the laboratory after collection, refer to FQM 7.3-1: Logistical Arrangement for Sample Submission.

Note: If swabs are not submitted to the laboratory immediately for analysis after sampling, store the swabs in the 5°C ± 3°C fridge for not more than 24hrs viability period.

9. Transport Conditions

9.1. Procedure

- 9.1.1. For liquid samples that have to be transported, leave an air space of about 5% of container capacity to allow for thermal expansion and shaking.
- 9.1.2. Water, chilled food, swab, air quality plates and food samples should be transported in clean and dry cooler box containing enough ice bricks to maintain temperature of cooler box between 0 -10°C (Ref: epa.vic.gov.au; Codex Alimentarius 2009) with the exception of retort water, bottled water and process food products (Canned food products, dry food parcels etc.).
- 9.1.3. Retort water may be received at high temperature as it is the water drained after sterilization/ pressurized cooking of canned food products.
- 9.1.4. Frozen samples should be transported in clean and dry cooler box containing enough ice bricks to maintain temperature of cooler box below 10°C in the cooler box and should be received in frozen state.
- 9.1.5. Samples should be properly sealed and should never come into direct contact with the ice or ice bricks.

10. Receipt of samples

10.1. Procedure

- 10.1.1. Sample receipt times to be met upon submission of samples is specified in **Table 1** below.

Table 1: Sample Receipt Times

Water, Ice & Air quality plates	Frozen food	Chilled food	Swabs
Monday – Friday 08:00-13:00	Monday – Friday 08:00-13:00 Note: samples to be analysed for Vibrio spp and E.coli should be submitted by 09H00, late submission will result in analysis the following day.	Monday – Thursday 08:00-13:00 Note: samples to be analysed for Vibrio spp and E.coli should be submitted by 09H00. late submission will result in analysis the following day or samples rejected for analysis.	Monday – Thursday 08:00-13:00 Note: samples to be analysed for Vibrio spp. Feacal coliforms and E.coli should be submitted by 09H00. late submission will result in analysis the following day or samples rejected for analysis.

- 10.1.2. Samples shall at all times be handed over to microbiology laboratory staff members.

- 10.1.3. Samples should be accompanied by a NSI test request form for microbiology FQM 4.4-2 and a purchase order.

11. Sample conditions

- 11.1. Temperature of the cooler box shall be taken upon arrival of samples with a calibrated infra-red thermometer. The receiving staff member shall assess the sample condition and record it on FQM 4.4-2 Test request form for microbiology.
- 11.2. Sample conditions to be met upon receipt of samples is specified in **Table 2** below and in Section.

Table 2: Conditions set by the laboratory for water, swab, fish and food products

Water samples	Swab samples and Air quality settled plates	Fish, Fishery product, shellfish, and food products	Milk and other dairy products
<p>Date collected: should not exceed: Water :24 hour period from sample collection if the sample was appropriate kept cool (0-10°C)</p> <p>Ice: maintain temperature of cooler box below 10°C in the cooler box and should be received in frozen state.</p> <p>Within 6 hours of sampling if the sample was transported without appropriate cooling</p> <p>(If the sampling time or date sampled is not given the analyst shall enquire and record on the date & sampling time)</p>	<p>Date collected: Same day not exceeding 24hrs from time of sample collection. (0-10°C)</p> <p>Within 6 hours of sampling if the sample was transported without appropriate cooling)- Note: -Air quality (AQ) settle plates shall not be submitted without a cooler box and Ice bricks. - Tolerance time to return AQ settle plates to the laboratory: 3 days</p> <p>-AQ settle plates: to be transported inverted</p> <p>(If the sampling time or date sampled is not given the analyst shall enquire and record on the date & sampling time)</p>	<p>Date collected: - Chilled/fresh/live products samples should not exceed 24hrs with cooling from time of sampling. (0-10°C)</p> <p>Within 6 hours of sampling if the sample was transported without appropriate cooling).</p> <p>-Frozen samples can be accepted as long as the product is received in a frozen state.</p> <p>Dry food products such as (Canned food products, dry food parcels etc.) no cooling required</p> <p>(If the sampling time or date sampled is not given the analyst shall enquire and record on the date & sampling time)</p>	<p>Date collected: Same day not exceeding 24hrs from time of sample collection. (0-10°C)</p> <p>Within 6 hours of sampling if the sample was transported without appropriate cooling)</p> <p>(If the sampling time or date sampled is not given the analyst shall enquire and record on the date & sampling time)</p>
Mode of transport:	Mode of transport:	Mode of transport:	Mode of transport:

<p>Cooler box (clean & dry)</p> <p>On ice bricks</p> <p>If not cooled then analyst to confirm if it was delivered within 6 hours of sampling time.</p>	<p>Cooler box (clean & dry)</p> <p>On ice bricks</p> <p><u>Swabs</u> If not cooled then analyst to confirm if it was delivered within 6 hours of sampling time</p>	<p>Cooler box (clean & dry)</p> <p>On ice bricks</p> <p><u>Chilled samples</u> If not cooled then analyst to confirm if it was delivered within 6 hours of sampling time.</p>	<p>Cooler box (clean & dry)</p> <p>On ice bricks</p> <p>If not cooled then analyst to confirm if it was delivered within 6 hours of sampling time</p>
<p>Sample containers:</p> <p>Sterile containers provided by the laboratory whether autoclaved or purchased as sterilised bottles.</p>	<p>Sample containers</p> <p>Closed/sealed in plastic packaging</p> <p>- In addition AQ settle plates to be sealed off with sufficient parafilm</p> <p>to prevent contamination</p>	<p>Sample containers:</p> <p>Closed/sealed packaging that will prevent contamination</p>	<p>Sample containers</p> <p>Closed/sealed packaging that will prevent contamination</p>
<p>Sample Label:</p> <p>- Clear & legible, and corresponds with what appears on the test request form.</p> <p>- Returned with traceability issued by laboratory</p>	<p>Sample Label:</p> <p>-Clear & legible, and corresponds with what appears on the test request form.</p> <p>-Returned with traceability issued by laboratory</p>	<p>Sample Label:</p> <p>- Clear & legible and corresponds with what appears on the test request form.</p> <p>-Returned with traceability issued by laboratory (sampling bag)</p>	<p>Sample Label:</p> <p>-Clear & legible, and corresponds with what appears on the test request form.</p>

11.3. Sample handling

11.3.1. Samples shall be handled in such a way that it doesn't deteriorate or become contaminated.

11.4. Sample Protection and Storage

11.4.1. Storage of all water, ice, milk, air quality plates, swab or chilled samples shall be done at **5± 3°C** if immediate analysis is not possible. Storage time should not exceed 24 hours before sample is analysed.

11.4.2. Storage of all frozen samples shall be kept in the freezer of **≤ -18.0 °C**, designated for samples to be analysed, if immediate analysis is not possible. Where possible, samples should always be analysed promptly by the laboratory.

12. References

- 12.1. ED 541: ISO 19458:2006 Water quality- Sampling for microbiological analysis
- 12.2. ED213: SANS 5763: Efficacy of cleaning plant, equipment and utensils, as well as swab technique.

- 12.3. ED 294: Industrial wastewater guidelines, Sampling and analysis of wastewaters, soils and wastes, IWRG701-June 2009, epa-vic.gov.au. EPA Victoria.
- 12.4. ED 411: Water Sample Collection and Handling Information, Water Microbiology Department. IG Micro-Med Environmental Inc. V6V-1H4.
- 12.5. ED 412: Standard Methods for the Examination of Water and Wastewater, American Water Works Association.
- 12.6. ED 420: VC 8031:10.14 Compulsory Specification for Frozen Shrimps (Prawns), langoustines and crabs. Test for efficacy of cleaning and disinfection of plant, equipment and utensils. Published by Government Notice 326 (Government Gazette 10614) of February 1987.