

# DMG MICROLABS

## SECTION 4 MICROBIOLOGICAL TESTING

### References

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Standard Methods for the Examination of Water and Wastewater, Mary Ann H. Franson (Editor), American Public Health Association American Water Works Association Water Environment Federation, 19<sup>th</sup> Edition, 1995, p9-17 to 9-20

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Stuttard, E. J., Jenson, I and Best, J. 1997. Sampling for microbial analysis, *In*, Foodborne microorganisms of public health significance, Ailsa D. Hocking, Glenda Arnold, Ian Jenson, Ken Newton and Peter Sutherland (Editors), 5<sup>th</sup> edition, AIFST NSW Branch Food Microbiology Group. Chapter 2, p19-29.

### 4.1 Introduction

Collecting samples for microbiological testing has its own unique set of problems. In addition to ensuring that the sample is representative of the matrix being sampled, the sample must be collected aseptically. This is to ensure that the samples are not contaminated by any sampling equipment, any sampling consumables such as diluent, or by the surrounding environment. It is also necessary to prevent the matrix being sampled from contaminating the surrounding environment.

There are four major sample types viz., air, water, food and surfaces. The detailed sampling procedures for each of these types are detailed below.



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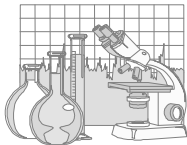
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## 4.2 Air sampling

This procedure covers air sampling for airborne microbiological contaminants in both indoor and outdoor air. It is suitable for all viable airborne bacteria and fungi. The procedure is based on impingement of airborne microorganisms in a gel coating of a filter. The gel is then dissolved, diluted and used to inoculate agar plates, which are incubated and counted to determine the airborne concentration.

### 4.2.1 Indoor Air Sampling

Check the Sartorius MD8 Air Sampling Pump draws air through the sampling port when turned on. Connect the sampling hose and check that a vacuum is set up when the open end of the sampling hose is blocked, to check there are no leaks in the hose. Clean the sampling head of the hose with water, then swab with 70% alcohol or methanol and allow to evaporate.

Check the "Air Sampling" briefcase against Checklist WS0003 to ensure that all of the required equipment and consumables are present. If they are present, sign the checklist. If items are missing, collect the necessary items and then sign the checklist. If items are missing and no replacements are available, discuss suitable alternatives with the Laboratory Manager or the Senior Microbiologist. Once an alternative has been agreed and placed in the crate, note the details of the alternative on the checklist and both the sampling staff member and the Laboratory Manager or Senior Microbiologist are to sign the checklist. Collect an appropriate size esky and sufficient frozen ice bricks/packs.

Unless the sampling site is well known to you, ensure you at least have a copy of the relevant pages of the UBD. Organise a vehicle and mobile phone, load the vehicle and depart the laboratory in sufficient time to reach the sampling site at the time agreed with the client.

Introduce yourself to the client and request any last minute instructions. It is preferable if the client or a representative remains with you during the sampling. Complete any formalities such as "signing in".

Find a suitable location for the pump and set up. Make sure the power leads do not cause any safety concerns (eg. tripping hazard in a room or corridor). Attach the hose and position the sampling end at the height required. This will normally be at head height, unless specifically requested by the client to sample the exit air from the duct outlet.

Aseptically remove filter from its plastic bag and aseptically attach it to the sampling head.

Set the pump to 8 minutes at  $0.8 \text{ m}^3/\text{h}$  and turn on. Enter the sample details on the sample analysis form.

As soon as the pump turns off, aseptically remove the filter and replace into the original bag.

Label the bag with the appropriate information, which shall contain at least: Client name and any other client specified identification; detailed location (street address); and Date and Time sampled. Place the sample(s) in the esky with ice brick(s).



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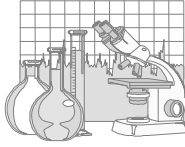
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Repeat until all the samples have been collected, labelled and placed in the esky.

Load up the vehicle and complete any formalities for leaving the site, such as signing out. Leave the site and transport the sample to the laboratory. Log the samples in to the laboratory for testing.

Note in the laboratory diary that the sampling has been completed and sign the diary entry.



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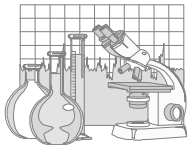
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## 4.3 Water sampling

This procedure covers potable, process, trade waste and effluent, and environmental and recreational water sampling. It is suitable for all water samples for subsequent testing by dilution plate count, MPN and membrane filtration techniques. It is emphasised that water samples must reach the laboratory, be logged in and testing commenced as soon as practicable within 24 hours.

### 4.3.1 Potable water sampling

Check the "Water Sampling" briefcase against Checklist WS0001 to ensure that all of the required equipment and consumables are present. If they are present, sign the checklist. If items are missing, collect the necessary items and then sign the checklist. If items are missing and no replacements are available, discuss suitable alternatives with the Laboratory Manager or the Senior Microbiologist. Once an alternative has been agreed and placed in the crate, note the details of the alternative on the checklist and both the sampling staff member and the Laboratory Manager or Senior Microbiologist are to sign the checklist. Collect an appropriate size esky and sufficient frozen ice bricks/packs.

Unless the sampling site is well known to you, ensure you at least have a copy of the relevant pages of the UBD. Organise a vehicle and mobile phone, load the vehicle and depart the laboratory in sufficient time to reach the sampling site at the time agreed with the client.

Introduce yourself to the client and request any last minute instructions. It is preferable if the client or a representative remains with you during the sampling. Complete any formalities such as "signing in".

Visually check the cleanliness of the tap mouth. If it is dirty, clean it with water and cotton wool. When visually clean, use fresh cotton wool swabs to dab alcohol onto the outer and inner surface of the tap mouth. Light the alcohol *with the other hand* and allow the alcohol to burn off which flame sterilises the surfaces.

Turn on the tap and allow it to run for at least two (2) minutes. Hold the sample jar on around the sides and carefully unscrew the lid and remove it just before placing the opened jar into the water stream. Fill the jar to within a few mm of the brim **BUT DO NOT ALLOW IT TO OVERFLOW.** Replace the lid and tighten to finger tight to ensure no leakage during transport.

Invert the jar two or three times to mix in the thiosulphate. Label the jar with the appropriate information, which shall contain at least: Client name and any other client specified identification; Detailed location (street address); and Date and Time sampled. Place the sample(s) in the esky with ice brick(s). Fill out the sample analysis form with the sample(s) details.

Load up the vehicle and complete any formalities for leaving the site, such as signing out. Leave the site and transport the sample to the laboratory. Log the samples in to the laboratory for testing.

Note in the laboratory diary that the sampling has been completed and sign the diary entry.



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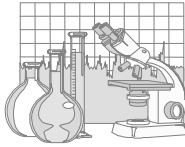
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## 4.3.2 Process water sampling

Check the “Water Sampling” briefcase against Checklist WS0001 to ensure that all of the required equipment and consumables are present. If they are present, sign the checklist. If items are missing, collect the necessary items and then sign the checklist. If items are missing and no replacements are available, discuss suitable alternatives with the Laboratory Manager or the Senior Microbiologist. Once an alternative has been agreed and placed in the crate, note the details of the alternative on the checklist and both the sampling staff member and the Laboratory Manager or Senior Microbiologist are to sign the checklist. Collect an appropriate size esky and sufficient frozen ice bricks/packs.

Unless the sampling site is well known to you, ensure you at least have a copy of the relevant pages of the UBD. Organise a vehicle and mobile phone, load the vehicle and depart the laboratory in sufficient time to reach the sampling site at the time agreed with the client.

Introduce yourself to the client and request any last minute instructions. It is preferable if the client or a representative remains with you during the sampling. Complete any formalities such as “signing in”.

Visually check the cleanliness of the sampling point/tap mouth. If it is dirty, clean it with water and cotton wool.

When visually clean, use fresh cotton wool swabs to dab alcohol onto the outer and inner surface of the tap mouth. If site safety regulations allow, light the alcohol *with the other hand* and allow the alcohol to burn off which flame sterilises the surfaces. If site safety regulations prohibit naked flames, allow the alcohol to evaporate.

Turn on the tap and allow it to run for at least two (2) minutes. Hold the sample jar on around the sides and carefully unscrew the lid and remove it just before placing the opened jar into the water stream. Fill the jar to within a few mm of the brim **BUT DO NOT ALLOW IT TO OVERFLOW.** Replace the lid and tighten to finger tight to ensure no leakage during transport.

Invert the jar two or three times to mix in the thiosulphate. Label the jar with the appropriate information, which shall contain at least: Client name and any other client specified identification; Detailed location; and Date and Time sampled. Place the sample(s) in the esky with ice brick(s). Fill out the sample analysis form with the relevant details.

Load up the vehicle and complete any formalities for leaving the site, such as signing out. Leave the site and transport the sample to the laboratory. Log the samples in to the laboratory for testing.

Note in the laboratory diary that the sampling has been completed and sign the diary entry.



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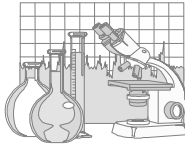
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## 4.3.3 Trade waste and effluent sampling

Check the “Water Sampling” briefcase against Checklist WS0001 to ensure that all of the required equipment and consumables are present. If they are present, sign the checklist. If items are missing, collect the necessary items and then sign the checklist. If items are missing and no replacements are available, discuss suitable alternatives with the Laboratory Manager or the Senior Microbiologist. Once an alternative has been agreed and placed in the crate, note the details of the alternative on the checklist and both the sampling staff member and the Laboratory Manager or Senior Microbiologist are to sign the checklist. Collect an appropriate size esky and sufficient frozen ice bricks/packs.

Unless the sampling site is well known to you, ensure you at least have a copy of the relevant pages of the UBD. Organise a vehicle and mobile phone, load the vehicle and depart the laboratory in sufficient time to reach the sampling site at the time agreed with the client.

Introduce yourself to the client and request any last minute instructions. It is preferable if the client or a representative remains with you during the sampling. Complete any formalities such as “signing in”.

If there is a designated sampling point from a pipe or tap, visually check the cleanliness of the sampling point/tap mouth. If it is dirty, clean it with water and cotton wool. If the sample is to be collected by the indirect technique by dipping a container into the water to be sampled, ensure the container is at least visually clean, although sterile is preferable.

When visually clean, use fresh cotton wool swabs to dab alcohol onto the outer and inner surface of the tap mouth. If using a non-sterile container to collect a large volume of sample from the water, rinse the container with alcohol. If site safety regulations allow, light the alcohol *with the other hand* and allow the alcohol to burn off to flame sterilise the surfaces. If site safety regulations prohibit naked flames, allow the alcohol to evaporate. There is no need to alcohol rinse if using a sterile container to collect a large volume of sample.

For a designated sampling point/tap, turn on the tap and allow it to run for at least two (2) minutes. If using a container to collect a large volume of sample, fill the container by placing it horizontally into the water (ie. on its side) and with the bottom of the container towards the direction of flow (Figure 4.3.1) so that the water enters the mouth of the container without first flushing it.

If sampling directly from the water flow hold the sample jar on around the sides and carefully unscrew the lid and remove it just before placing the opened jar into the water stream. Fill the jar to within a few mm of the brim **BUT DO NOT ALLOW IT TO OVERFLOW.** Replace the lid and tighten to finger tight to ensure no leakage during transport. If filling the sample jar from the large volume container, carefully remove the sample jar lid and fill the jar without overflowing. Replace the lid and tighten t finger tight.

Invert the jar two or three times to mix in the thiosulphate. Label the jar with the appropriate information, which shall contain at least: Client name and any other client specified identification;



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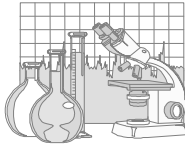
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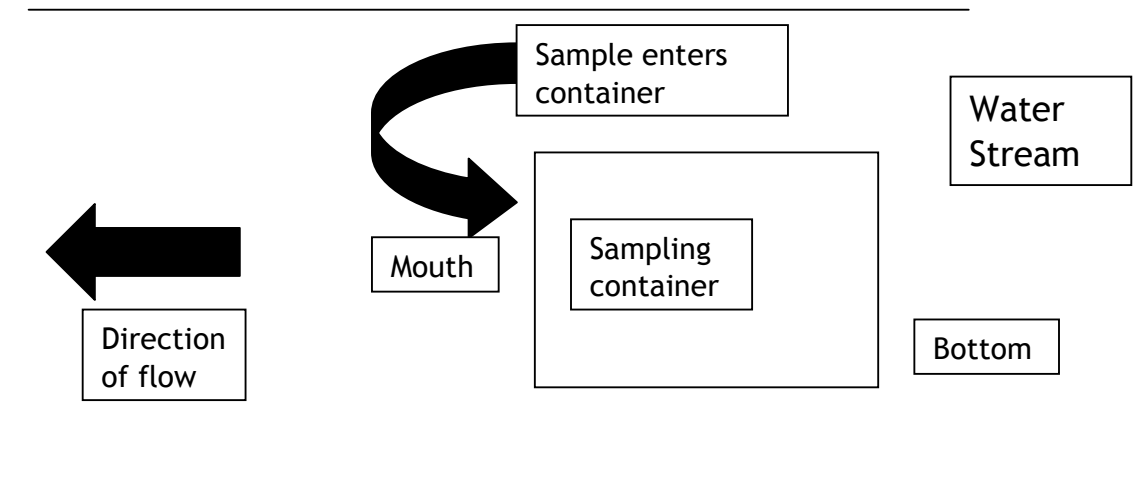
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Detailed location; and Date and Time sampled. Place the sample(s) in the esky with ice brick(s). Fill out the sample analysis form with the relevant details.

**Figure 4.3.1 Collection of water samples from flowing streams**



Load up the vehicle and complete any formalities for leaving the site, such as signing out. Leave the site and transport the sample to the laboratory. Log the samples in to the laboratory for testing.

Note in the laboratory diary that the sampling has been completed and sign the diary entry.



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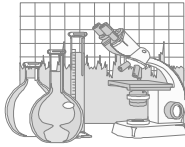
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## 4.3.4 Environmental and recreational water sampling

Check the “Water Sampling” briefcase against Checklist WS0001 to ensure that all of the required equipment and consumables are present. If they are present, sign the checklist. If items are missing, collect the necessary items and then sign the checklist. If items are missing and no replacements are available, discuss suitable alternatives with the Laboratory Manager or the Senior Microbiologist. Once an alternative has been agreed and placed in the crate, note the details of the alternative on the checklist and both the sampling staff member and the Laboratory Manager or Senior Microbiologist are to sign the checklist. Collect an appropriate size esky and sufficient frozen ice bricks/packs.

Unless the sampling site is well known to you, ensure you at least have a copy of the relevant pages of the UBD. Organise a vehicle and mobile phone, load the vehicle and depart the laboratory in sufficient time to reach the sampling site at the time agreed with the client.

Introduce yourself to the client and request any last minute instructions. It is preferable if the client or a representative remains with you during the sampling. Complete any formalities such as “signing in”.

The sample is to be collected directly by dipping the sample jar into the water, or indirectly by dipping a container into the water to be sampled. If the indirect collection procedure is used, ensure the container is at least visually clean, although sterile is preferable.

For the indirect collection technique, if using a non sterile container to collect a large volume of sample from the water, rinse the container with alcohol. If site safety regulations allow, light the alcohol and allow the alcohol to burn off to flame sterilise the surfaces. If site safety regulations prohibit naked flames, allow the alcohol to evaporate. There is no need to alcohol rinse if using a sterile container to collect a large volume of sample, or if the direct collection technique is used.

Fill the container by placing it horizontally into the water (ie. on its side) and with the bottom of the container towards the direction of flow (Figure 4.3.1) so that the water enters the mouth of the container without first flushing it.

If sampling directly from the water flow hold the sample jar on around the sides and carefully unscrew the lid and remove it just before placing the opened jar into the water stream. Fill the jar to within a few mm of the brim **BUT DO NOT ALLOW IT TO OVERFLOW.** Replace the lid and tighten to finger tight to ensure no leakage during transport. If filling the sample jar from the large volume container, carefully remove the sample jar lid and fill the jar without overflowing. Replace the lid and tighten to finger tight.

Invert the jar two or three times to mix in the thiosulphate. Label the jar with the appropriate information, which shall contain at least: Client name and any other client specified identification; Detailed location; and Date and Time sampled. Place the sample(s) in the esky with ice brick(s). Fill out the sample analysis form with the relevant details.



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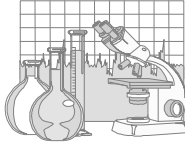
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Load up the vehicle and complete any formalities for leaving the site, such as signing out. Leave the site and transport the sample to the laboratory. Log the samples in to the laboratory for testing.

Note in the laboratory diary that the sampling has been completed and sign the diary entry.



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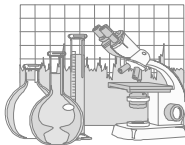
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## 4.4 Surface Swab Sampling of Equipment, Surfaces, Air-handling Units and Ductwork, etc.

### 4.4.1 Introduction

This technique is used for assessing the cleanliness or the extent of contamination of surfaces (e.g. equipment, air conditioning ducts, floors, drains, and food surfaces). A sterile 3M Quikswab is preferred as it is a self-contained sampling system. The swab is used as per the current version of the 3M instruction booklet.

If no 3M swab is available, then use a sterile swab, moistened in a sterile recovery diluent, is repeatedly brushed over a defined surface area, then thoroughly rinsed in the recovery diluent and the diluent used to inoculate appropriate growth media.

### 4.4.2 Scope

The technique is suitable for a general assessment with a non-selective growth medium such as for standard plate counts, and can be adapted for specific organisms such as coliforms and *E. coli* by using appropriate enrichment broth and selective growth media. The area selected should be representative of the surface, and sufficiently large to be statistically significant. It can be used for assessing the microbiological condition of most surfaces, particularly in food preparation/ processing areas, food preparation tools and clothing, and air conditioning ducts.

### 4.4.3 Procedure

Check the "Surface Swab Sampling" briefcase against Checklist WS0002 to ensure that all of the required equipment and consumables are present. If they are present, sign the checklist. If items are missing and no replacements are available, discuss suitable alternatives with the Laboratory Manager or the Senior Microbiologist. Once an alternative has been agreed and placed in the crate, note the details of the alternative on the checklist and both the sampling staff member and the Laboratory Manager or Senior Microbiologist are to sign the checklist. Collect an appropriate size esky and sufficient frozen ice bricks/packs.

Unless the sampling site is well known to you, ensure you at least have a copy of the relevant pages of the UBD. Organise a vehicle and mobile phone, load the vehicle and depart the laboratory in sufficient time to reach the sampling site at the time agreed with the client.

Introduce yourself to the client, ensure you have interpreted any sampling details supplied by them correctly and request any last minute instructions. It is preferable if the client or a representative remains with you during the sampling. Complete any formalities such as "signing in".

Select the item(s) and surface(s) to be swabbed, and then select a representative area of the surface.



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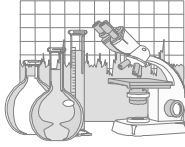
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Dip the template (the part which will be placed on the surface) in alcohol and if the site safety procedures allow, light the alcohol and allow it to burn off. If the site safety procedures do not allow naked flames, simply allow the alcohol to evaporate.

Place the template on the surface. For transport tubes follow the instructions on the individual packets. For dry swabs and peptone water remove a swab from its container and aseptically moisten the cotton wool tip. Open the sterile swab packet at the stick end, being careful not to touch any portion which might be inserted into the bottle of recovery diluent, and remove aseptically.

Open bottle of maximum recovery diluent (MRD), moisten swab head and press out excess solution against interior wall of bottle, with a rotational motion.

Rub the swab head slowly and thoroughly over the required surface area, rubbing the swab three times over this surface, reversing direction between successive strokes.

Return the swab head to the solution bottle and break or cut the stick leaving the swab head in the bottle. Replace the screw cap. Label the jar with the appropriate information, which shall contain at least: Client name and any other client specified identification; Detailed location; and Date and Time sampled. Place the sample(s) in the esky with ice brick(s). Fill out the sample form with the relevant details.

Load up the vehicle and complete any formalities for leaving the site, such as signing out. Leave the site and transport the sample to the laboratory.

Log the samples in to the laboratory for testing. Note in the laboratory diary that the sampling has been completed and sign the diary entry.



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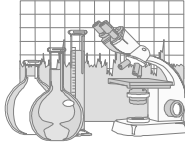
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## 4.5 Food Sampling

Food covers a multitude of sample matrices, from liquids (which range from aqueous to high viscosity liquids), through to solids (ranging from blocks such as cheese to powders, meat and meat products, to fruit and vegetables). The sample obtained must be representative of the main bulk of the food matrix. All samples must be obtained and processed aseptically. Instruments such as tongs or forceps, knives, scissors, or scalpel blades should be wrapped and sterile until used, or alcohol sterilised between each sample.

### 4.5.1 Procedure

Check the "Food Sampling" briefcase against Checklist WS0004 to ensure that all of the required equipment and consumables are present. If they are present, sign the checklist. If items are missing, collect the necessary items and then sign the checklist. If items are missing and no replacements are available, discuss suitable alternatives with the Laboratory Manager or the Senior Microbiologist. Once an alternative has been agreed and placed in the crate, note the details of the alternative on the checklist and both the sampling staff member and the Laboratory Manager or Senior Microbiologist are to sign the checklist. Collect an appropriate size esky and sufficient frozen ice bricks/packs.

Unless the sampling site is well known to you, ensure you at least have a copy of the relevant pages of the UBD. Organise a vehicle and mobile phone, load the vehicle and depart the laboratory in sufficient time to reach the sampling site at the time agreed with the client.

Introduce yourself to the client, ensure you have interpreted any sampling details supplied by them correctly and request any last minute instructions. It is preferable if the client or a representative remains with you during the sampling. Complete any formalities such as "signing in".

All of the samples are to be obtained aseptically, with individual disposable sterile sampling implements. If reusable sampling implements are used, they should be flame sterilised, or if safety regulations do not permit naked flames, sanitise with a solution of 100 mg/L free available chlorine (or equivalent). Sample containers must be sterile. Label the container with the appropriate information, which shall contain at least: Client name and any other client specified identification; Detailed location; and Date and Time sampled. Place the sample(s) in the esky with ice brick(s). Fill out the sample form with the relevant details.

Once all of the required samples have been obtained load up the vehicle and complete any formalities for leaving the site, such as signing out. Leave the site and transport the sample(s) to the laboratory.

Log the samples in to the laboratory for testing. Note in the laboratory diary that the sampling has been completed and sign the diary entry.



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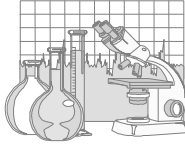
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# DMG MICROLABS

## 4.5.2 Food types

### Liquids

These are to be sampled by sterile pipette, with the vacuum supplied preferably by automatic pipettor, with hand operated bulb, or small pump as alternatives, depending on the viscosity of the liquid.

### Solid foods

***Foods such as cheese, butter and desserts are to be cored with a borer or trier, or sampled with a sterile spoon.***

### Frozen foods

A sterile drill, chisel or corer is to be used on solid frozen foods. Partly thawed foods may be able to be cored with a cheese trier or similar implement. For individually frozen small food particles (eg. frozen peas), use a sterile spoon.

### Chilled foods

The surfaces of chilled foods such as meat carcasses are sampled by swabbing. Aseptically add 10 mL sterile diluent to a sterile dry sponge swab and squeeze the sponge inside the bag to thoroughly moisten it. Select the three 100 cm<sup>2</sup> areas to be swabbed on the carcass. Alcohol sterilise the template and place it on the first area of the carcass. Open the bag, grasp the sponge at one end through the bag, pull the bag carefully back over your hand to expose the sponge, and swab the area by 3-5 strokes along the axis of the template, 3-5 strokes at right angles to the first set, and 3-5 circular motion strokes. Shift the template to the next area of the carcass and swab this area. Repeat for the third area of the carcass. Carefully pull the sponge bag back over the sponge, add the remaining 15 mL of diluent and squeeze the sponge to thoroughly mix the sponge and diluent. Care must be taken to prevent contamination of the inside of the bag during the time that the sponge is exposed.

Chilled foods may also be sampled by excision. Alcohol sterilise scissors or use a sterile scalpel blade to excise sufficient sample and aseptically transfer the sample to a sterile container.

### Dry foods

Dry foods are in powder or granulated form. Sampling of pre-packaged dried foods is simply a matter of collecting sufficient packets to provide the sampling weight. Sampling from bulk dried foods requires the compositing of several sub-samples from the bulk container, adequately mixing them and taking the sample for analysis from this mixture. Use disposable sampling implements or alcohol flame sterilise reusable implements (such as long handle spoons, or long handle "grabbers" as used to sample grain). Aseptically transfer the sample to a sterile container.



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