MANUAL FOR WATER QUALITY TESTING

MICS6 - DPRK - 2017

INTRODUCTION TO THE MANUAL

This Manual is intended for all MICS field staff and outlines the required steps that need to be taken during MICS data collection in order to accurately assess drinking water quality. Measurers in particular should carry these instructions with them in the field and review them regularly to make sure they are always following the correct procedures. Supervisors should also frequently refer to this Manual in the field when observing the work of measurers.

BACKGROUND ON WATER QUALITY TESTING

The objective of this water quality module is to obtain a nationally-representative view of the quality of water that people drink in their home and the quality of their drinking water source. In each cluster of the survey, a number of households will be randomly selected for *Thermotolerant Coliform (TTC)* testing. *TTC* is a faecal indicator bacteria, meaning that it is likely to be present when faeces or raw sewage has entered the water supply. The presence of *TTC* in drinking water does not necessarily mean that the person drinking it will become sick, but it indicates that over time the household is at a higher risk for waterborne diseases. The World Health Organization recommends as a guideline that there should be no *TTC* present in a 100 mL sample of water.

RESPONSIBILITIES OF FIELD TEAM MEMBERS DURING THE COLLECTION OF WATER QUALITY MEASUREMENTS

Measurers will be responsible for conducting *TTC* tests in the field, and for completing the water quality questionnaire. They will be responsible for maintaining the equipment and notifying Supervisors if equipment is faulty or short in supply.

Supervisors will verify the household selection for water quality testing in CAPI and share this with the measurers after reaching each cluster. They will be responsible for coordinating the work of the measurer by making sure he/she knows where to find the households where samples are to be collected at the source and in the home. Supervisors will advise measurers when they should visit the household and provide bottled water for the blank test when needed. The supervisors will be responsible for ensuring that measurements are taken following the exact steps and procedures outlined in this Manual. In situations where measurers are routinely making errors in taking and/or reading measurement, or in reporting the information on the questionnaire, the supervisor should consult with the fieldwork director and/or survey coordinator about corrective actions. Supervisors are also responsible for coordinating resupply of the MLSB bottles to the measures from the central coordination unit.

GENERAL PRECAUTIONS FOR MEASURERS

Carrying out microbiological analysis presents certain risks as it is highly likely that you will be handling equipment and materials that are potentially contaminated with harmful pathogens. This is especially relevant for the filter membranes, absorbent pads and petri dishes that are used in the test. For these reasons, general hygiene and aseptic procedures are of paramount importance and extra care must be taken when working in the field.

(1) Preventing contamination: aseptic technique

Care must be taken during sampling and testing to prevent contamination of the sample by bacteria in the environment or from previous water samples. Aseptic technique for field sampling can be summarized as follows:

- Always wash hands with soap or apply gel hand sanitizer before starting a new sample or touching equipment that will touch the sample.
- Sanitize any equipment that comes in contact with the sample. Petri dishes must be sterilised in the pressure cooker or using a flame while the filtration mechanism is disinfected using formaldehyde gas produced by the burning of methanol. Forceps are sterilised using a flame.

(2) Time management

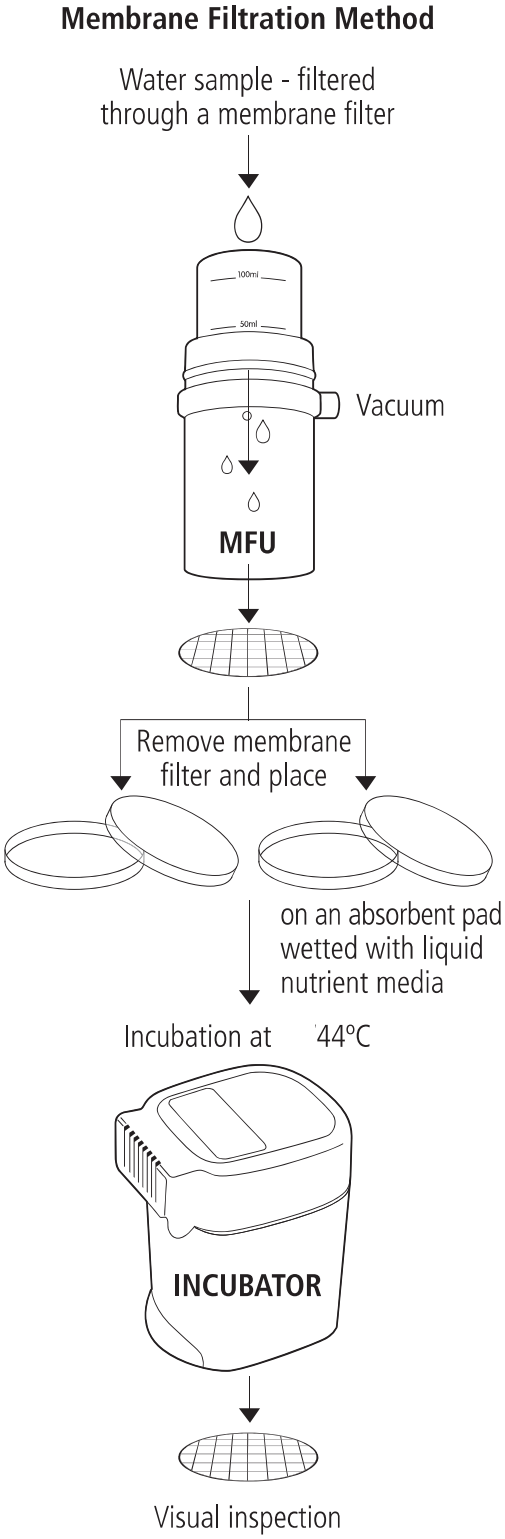
The actual water quality test itself requires approximately 30 minutes. However, the measurer must also plan time to visit the household's drinking water source and to read the sample results the next day. Results should be read after 16 to 36 hours of incubation after the test is completed.

(3) Transport of samples

In some cases, it may be more convenient to collect a sample and process it for testing at another location. In this case, short transit times (up to 30 minutes) are acceptable provided samples are kept out of direct sunlight. The sterile sample bags that have been provided must be used for such cases.

(4) Sample incubation

To provide the right conditions for *TTC* to grow into countable colonies, the petri dishes must be kept at 44 °C in an incubator for 16 to 36 hours. If the temperature is too low for an extended period of time, the *TTC* will grow too slowly to be visible and if the temperature is too high, the *TTC* might be killed or overtaken by other bacteria suited for the hotter conditions. It is important that petri dishes remain in the incubator, and that the incubator is



turned on and functioning for the duration of the incubation.

Figure 1 – TTC testing process

SUMMARY OF THE TEST

The Wagtech kits use classical laboratory techniques and equipment that have been adapted for use in the field. They conform fully with guidelines issued by WHO on accepted field-based methods for the microbiological analysis of drinking water. The testing of water samples for coliform bacteria uses a method called Membrane Filtration.

COLLECTION OF SAMPLES

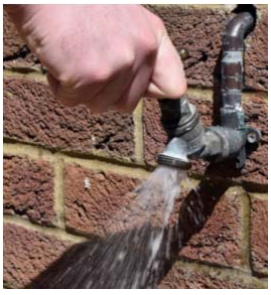
All samples will be collected only using a sterilized sample collection bags or directly into the metallic sample collection cup. Water testing is performed in accordance with the instructions in the questionnaire. The test may be performed at the household, or the measurer may perform the test at a more convenient location.

Household drinking water sampling

Since a main purpose of this part of the survey is to determine the quality of water as it is actually consumed, samples will be collected from household drinking water taken from the point of consumption. The measurer will ask the survey respondent for "a glass of water that members of your household usually drink".

Water source sampling

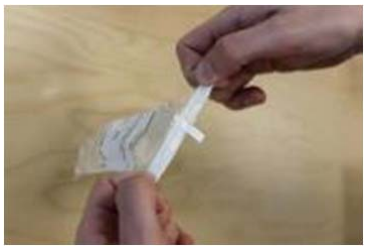
The water source should be determined based on the responses given by the household. When water samples are collected from the source, water should be flushed for 30 seconds whenever this is feasible. For example, a tube-well should be pumped for 30 seconds, or a tap should be opened for 30 seconds, before collecting the sample. If the water is collected from the source by hand (as in an unprotected spring or dug well with bucket), flushing is not necessary. If the water is being collected from a spring, stream or a river, the sample should be collected by facing the mouth of the container/bag towards the opposite direction of the flow.



Blank test sampling

Supervisors will provide the measurer with distilled or bottled water for the blank test. This will be a bottle of water known to be of high quality (without any bacteria). The blank test using the high quality water will be performed in the same way as water from the household or source.

Taking a sample with a sample collection bag

 **A.** Write sample code on sample collection bag per instructions in questionnaire	 **B. Sanitize hand**s then open the sample collection bag	 **C.** Collect the water sample in sample collection bag – ensuring that the level of the water is above the 110mL line
 **D.** Close the sample collection bag by rolling over the white tab	 **E.** Flip the sample collection bag 3 times	 **F.** Fold white tabs closed to seal the sample collection bag

Taking a sample with the Palintest sample cup

A. Rinse the sterilised metallic cup with water from the selected source, discard it, and then fill the cup with your sample. There is no labelling and the water should be taken for testing immediately.

CHECKLISTS

Field household interviews	*Guesthouse / hotel*
✓ Membrane filtration unit ✓ Incubator ✓ Vacuum pump ✓ Forceps ✓ 47mm membrane filters	✓ Dish soap ✓ Absorbent pads and tool ✓ Pressure cooker ✓ Portable stove

✓ MLSB media ✓ Distilled water ✓ Sterile pipettes ✓ Petri dishes containing absorbent pad ✓ Methanol ✓ Lighter ✓ Matches ✓ Paper towel ✓ Whirl-Pak sample bags ✓ Zip-lock plastic bags ✓ Hand sanitizer	

WATER QUALITY TESTING USING THE PALINTEST KIT

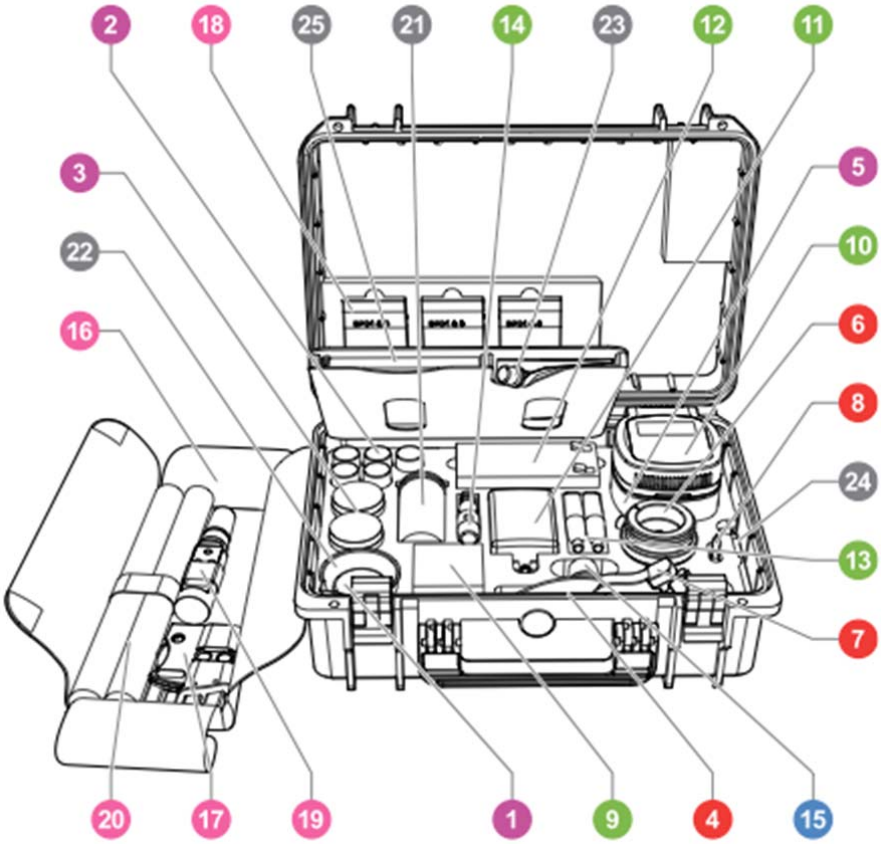


Figure 2: Equipment required for water quality testing

Preparing the media, petri dishes	(1) Membrane Lauryl Sulphate Broth (MLSB) (2) Media measuring device (3) Absorbent pads (4) Pad dispenser (5) Petri dishes (in incubator)

Membrane filtration	(6) Membrane filtration unit (7) Hand vacuum pump (8) Forceps (9) 47mm membrane filters
Incubation and incubator operation	(10) Incubator (11) Mains power supply (12) Battery (13) Battery leads (14) Car charging Lead
Microbiological results	(15) Hand lens

Performing the water quality test for TTC

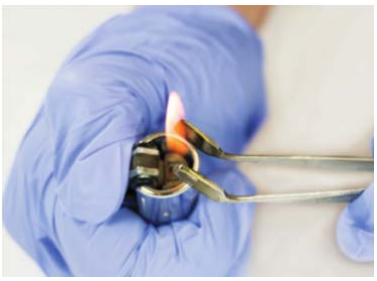
1. Disinfect your hands using sanitizer gel/solution

2. Sanitize the filtration apparatus. Using a pipette, add just a small amount (10-15 drops, 1mL) of methanol to the metallic sample cup and swirl it around to cover as much of the surface as possible. Hold the cup away from your body and your face, tip it on an angle, and using the lighter, move the flame towards the methanol coated surface. The methanol will ignite instantly and there will be a pale blue flame. Place the sampling cup base down on a flat stable surface.

3. Assemble the filter funnel and rubber base. Ensure the filter funnel is in the correct locking position before for the sterilisation procedure.

4. Wait for the flame in the cup to be extinguished. Then invert the filter funnel and base into the cup as shown. Leave it for 15 minutes as the formaldehyde gas disinfects the entire apparatus.

5. After 15 minutes, take out the filtration unit which is now sterile. Take the water sample using the sterilised metallic cup as needed.



6. Sterilise the forceps using a flame from a lighter and allow it to cool for a couple of seconds

7. Remove one membrane filter from box. Using the sterile forceps, carefully take the white membrane out of the sterile wrapping (careful not to touch with your fingers)

8. Place the sterile membrane gridded side up, onto the bronze membrane support. If the membrane tears, falls, or becomes contaminated discard it and use a new one. Place the forceps down on your workspace but inside the membrane filter wrapping to keep them sterile.

9. Lock the filtration unit into place in the correct position for the filtration process

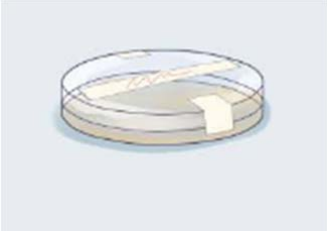
10. Using the requested glass of water, metallic sample cup or sample collection bag, pour the sample into the filtration unit up to the 100mL mark.

11. Suction the water through the membrane under vacuum pressure using a pistol-grip pump.

12. If the absorbent pads are not yet inside the petri dish, add a sterilised pad using the dispenser tool or sterilised

13. Using a new sterile pipette add the pre-prepared

	forceps. The dispenser tool should be sterilised using a little bit of methanol and clean paper towel prior to use.	MLSB to the absorbent pad.

 **14.** Ensure that the entire pad is saturated with MLSB	 **15.** Use the sterile forceps to remove the membrane from the filtration unit	 **16.** Place the membrane carefully onto the absorbent pad
 **12.** Close the petri dish. Place a sticky label on the dish lid, and clearly write the sample code Example code: H-012-03 Label codes: **1st letter:** H = household sample, S = source sample, B = blank test **NUMBERS: CLUSTER + HOUSEHOLD**	 **13.** Store the petri dish(s) safely and securely inside the incubator. Repeat the entire process for any additional tests at the household and for any additional households.	 **14.** Begin the incubation cycle. Ensure that the temperature is set to 44 degrees Celsius. Make sure that the battery is fully charged and always use a power supply (see options below) if one is available. Incubate all petri dishes for 16 to 36 hours before stopping the incubation and reading the results.

14. Collect all garbage and dispose of properly; show respect to households and do not leave behind any materials. Trash will be incinerated periodically. Alternatively, it can also be disinfected with bleach.

Charging the incubator

The incubator has its own internal battery power source which should last for 4 or 5 full incubations, however it must be charged regularly. There are several options for charging the incubator, based on the power sources available:

A. To mains power

B. To a rechargeable battery

C. To a vehicle battery

D. To a car lighter socket

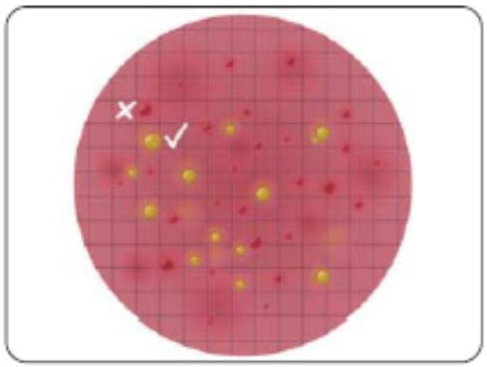
It is important to use every opportunity to recharge the incubator battery. Do not wait for the battery to be empty before charging!

INTERPRETING RESULTS

General guidelines for incubation and interpretation of TTC results:

- Ensure that the incubator was functional for the entire incubation cycle – either on battery or other power source
- Make sure that the incubator was always set to 44 degrees Celsius.
- Read results after 16 to 36 hours of incubation.

Reading Results

	**A.** After incubation, remove the petri dishes from the incubator. Microbiological test results are determined by counting the growth of bacterial colonies. If the colonies are difficult to see, use the hand lens included with the kit. Reading and interpretation immediately after incubation.
	**B.** All ***yellow*** colonies with a size >1mm should be counted. Other bacteria may grow and form colonies that are of different colours. These are not *TTC* and should not be counted
	**C.** Follow the grids left-right and row-by-row to count the total number of yellow colonies >1mm. In case there are many colonies on a plate, the number of colonies in one quarter of the plate may be counted, and this number multiplied by four. If there are more than one hundred colonies on the membrane, the result can simply be recorded as "101". If the bacteria levels are very high, no individual colonies may be seen, but the entire plate may turn yellow. In this case, the result should also be recorded as "101". If for any reason it is not possible to interpret the results or incubation could not be completed this should be recorded as "998". All results are recorded onto the water quality questionnaire.

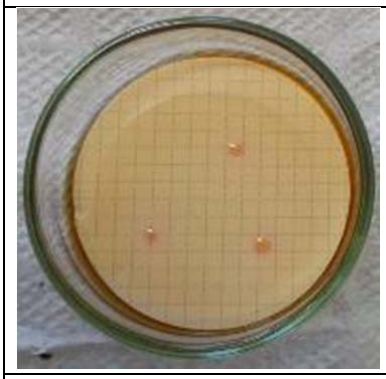
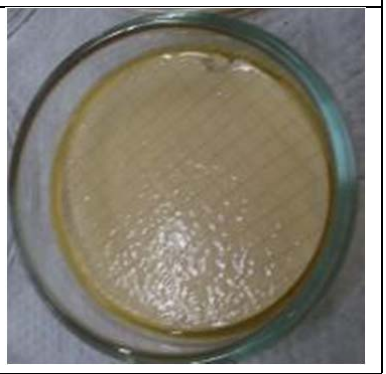
		
Number of *TTC* colonies : 0	Number of *TTC* colonies : 101	Number of *TTC* colonies: 9
		
Red or pink colonies do not signify contamination Number of *TTC* colonies : 0	Brown color does not signal contamination. Number of *TTC* colonies : 0	A yellow shiny indicates many, many colonies. Number of *TTC* colonies : 101

Figure 3: Examples of different situations you might encounter with your plates

STERILISATION OF THE USED PETRI DISHES, PADS, AND FILTERS

Petri dishes, absorbant pads, and membrane filters that have already been used are a potential health risk. Therefore they need to be handled, cleaned, and sterilised properly. Used pads and membranes will be placed in a bio-hazard ziplock bag and must be incinerated or disinfected with bleach. Petri dishes can be re-used but must first be washed and sterilised in a pressure cooker or with a flame (as with forceps).

	1. Wash the petri dishes with some soap and water, and let them dry. Find a suitable location to operate the portable gas stove. Add sufficient water to the pressure cooker. Place the petri dishes secured in a stack as shown, on top of a rack inside the pressure cooker. Secure the lid of the pressure cooker.

	2. Turn on the gas stove.
	3. Heat the pressure cooker to full pressure. Monitor the pressure cooker, and once the first burst of steam is released, wait another 15 minutes to complete sterilisation. After sterilisation, turn off the stove and allow the pressure cooker to cool before removing the petri dishes.
	4. Sterilise the pad dispenser tool by adding a few drops of methanol to the contact area and wiping clean with a paper towel. Using the tool, add a sterilised pad to each petri dish. Alternatively, you can add the pads in the field before each test.
	5. Make sure the petri dishes are securely stored back inside the Palintest incubator or inside a clean ziplock bag, until they are needed for use.

PREPARATION OF THE MLSB MEDIA

MLSB contains the food that the bacteria will need to grow into colonies so we can count them. The MLSB will be prepared centrally and each team of measurers will receive 5 bottles of 25mL of MLSB media – sufficient for a total of 50 tests. If you begin using your last bottle of MLSB, return your used MLSB bottles to your supervisor so that they can secure additional quantities.

Items required to produce MLSB media:

- Membrane Lauryl Sulphate powder
- Washed and dry Palintest MLSB bottles

- Distilled water
- Pressure cooker
- Portable stove

	1. Unscrew the blue lid/spoon of the MLSB bottle. Precisely add 10x level spoonsful of MLSB powder from the container
	2. Pour 100mL of distilled water into the MLSB bottle (up to the lowest lip of the bottle).
	3. Screw on the lid tightly and shake the MLSB bottle to dissolve all the powdered MLSB. Once fully dissolved, the colour of the liquid will be bright red/pink. Repeat the process for as many MLSB bottles as you need to produce.
	4. For each of the MLSB bottles, slightly unscrew the lids so that they remain fixed but are a little bit loose (this is to ensure air can come and go during pressurised sterilisation. Gently place the MLSB bottles into the pressure cooker. Then follow the same instructions as above for sterilisation using a pressure cooker.

Once produced, the MLSB in the bottles should be stable for 6 months if stored in a refrigerator. If not stored in a refrigerator, it can be stored for up to 3 months if kept in a dark and dry location. Periodically check the bottles for signs of contamination (such as yellow colour or cloudiness) and discard and reproduce as necessary.

USEFUL TIPS AND TRICKS

- Methanol is highly flammable so store and handle it carefully
- Always wash your hands and use hand sanitizer before conducting analysis
- Never eat, drink, or smoke while conducting the test

- Never directly touch any contaminated surfaces (filter, absorbant pad, dirty petri dish)
- Ensure a clean workspace as best as you can under field conditions
- Never dispose of materials directly into the environment – store in the ziplock bag and dispose of by incineration or disinfection with bleach
- Seven components must always be kept sterile throughout the testing process
    - Metallic sample collection cup
    - Membrane filtration unit
    - Petri dishes
    - Absorbent pads
    - Membrane filter
    - Forceps
    - MLSB media