Module 2 Script: GTFCC recommendations. SAMPLE COLLECTION, PREPARATION AND TRANSPORT FOR CHOLERA.  *V1.0 November 2024.*

Slide 1: GTFCC recommendations. SAMPLE COLLECTION, PREPARATION AND TRANSPORT FOR CHOLERA.

Welcome to the second module of this course entitled “Sample collection, Preparation and Transport for Cholera”.

Slide 2: Learning objectives.

At the end of this module, you will be able to:

* Describe what makes for an appropriate specimen for cholera testing
* Safely collect and prepare high quality samples for cholera testing
* Define the key factors affecting storage and transport of these samples
* Identify what is needed for adequate sample transport
* And apply best practices for reporting with the samples

Slide 3: Outline.

This module is divided into the five following sections.

Slide 4: SAMPLE COLLECTION.

Let us dive into our first section: Sample collection for cholera testing

Slide 5: The result of any laboratory examination is only as good as the sample received in the laboratory.

One key message to remember is that the result of any laboratory examination is only as good as the sample that was received by the laboratory. Think of it like this: can we cook a good meal if we only receive damaged eggs?

Slide 6: Outcomes of sample collection

Before any type of testing can be done, a good quality sample must be collected and sent to the laboratory.

In fact, if the sample collection and transport is done correctly, the outcomes will be:

* Accurate and reliable test results
* Test results can be reported in a timely way
* There may be high customer/patient satisfaction
* Costs are reduced
* And very importantly we can obtain the correct diagnosis and decide on effective treatment

Now if a sample is incorrectly collected and transported, there may be negative outcomes such as:

* We may not trust the lab results
* There may be delays in getting lab results
* There may be a need to re-test or even to take another sample from the unfortunate patient
* There may be decreased satisfaction on the side of the patient or the doctor
* It may cost more to re-test
* And very importantly, poor lab results can lead to incorrect diagnosis and ineffective choice of treatment for the patient or in worst case event, lead to injury or death

Slide 7: Specimen criteria for cholera testing.

To test a patient for cholera, we need to test the stool.

We can collect fresh, loose stool or perform a rectal swab.

The specimen (or sample) should be collected from patients who show symptoms for cholera, who are suspected cases of cholera and selected according to the testing strategy that is applied in your area. Refer module 1 introduction to cholera and cholera testing for more information on this.

It is preferable to collect a sample from the patient when the patient has been sick for less than 4 days. During the first four days of illness, there are usually more bacteria in the stool which helps the tests work better.

It is also preferable that the patient has not been given any antibiotics before the sample has been taken. Antibiotics may kill the bacteria in the stool and this may lead to increased difficulty in confirming the diagnosis of cholera.

Slide 8: Antibiotics.

Patient care should always be prioritized. Specimens from patients who have not taken antibiotics are preferred.

Again, antibiotics may kill the bacteria that is in the stool and any tests performed afterwards could then come out falsely negative.

It may happen that despite your efforts the patient has been given antibiotics. In this case it is very important to report this information to the laboratory in the sample collection form. It is important to note which antibiotics were taken, how much antibiotics were taken i.e the dosage and when was the antibiotic taken or for how long.

Saying that you have no information on the reporting form is also information by itself!

Slide 9: Safely collect samples.

Before starting the procedure, it is always important to remember the basic safety measures. These are key to keeping you and your community safe.

First, wear gloves every time you collect or handle a stool sample.

If you have any cuts or abrasions on your skin, if possible cover them with adhesive dressing or skin tape first.

When you are done collecting and handling the sample, remove the gloves and wash your hands with soap.

Use a new pair of gloves and wash your hands in between each patient. You may also protect your clothes by wearing scrubs or a lab coat.

Be sure to adhere to proper procedures for the disposal of any waste.

Slide 10: How to collect specimens for cholera testing.

To test for cholera we first need to collect a sample of stool from the patient.

If the patient can produce loose stool by themselves, we will collect the loose stool in a primary container before transferring some of it into a stool cup or onto a fecal swab.

In rare cases the patient may not be able to produce loose stool and collecting a rectal swab may be required. Rectal swabs are in fact rarely needed with cholera patients as these patients often have profuse diarrhea.

After further sample preparation, you may have other types of samples such as a sample on filter paper or in Alkaline Peptone water. This will be presented in the next section of this module.

Slide 11: All about bleach.

Before we look further into the steps to collect a sample, a word of caution. Bleach or other disinfectants can kill bacteria responsible for cholera in a sample. This may lead to the tests not working or not detecting cholera correctly. We would call that a false negative.

It is important to be careful to collect the stool from patients using only clean containers without any traces of disinfectant or detergent residue or other contaminants such as urine. Before use, bedpans and buckets should be washed, disinfected, rinsed thoroughly with clean water and dried.

Slide 12: Able patients.

How to collect loose stool.

Ideally, the patient is able to provide you a sample themself. In that case, provide the patient with a container such as a bucket or a bedpan without traces of detergent or disinfectant, or a new plastic bag (like a zip-lock bag) or ideally a wide biodegradable paper cup with a wide enough opening for them to use to collect loose stool.

Explain to the patient that they should urinate before using the container or the bag. They can then pass stool in the container or bag.

Slide 13: Bed-ridden patients.

If the patient is bed-ridden, use a clean, unused bedpan or bucket placed under the bed or under the patient. Again, make sure the bedpan does not have any traces of bleach or other disinfectant.

Slide 14: To transfer stool to a stool cup.

You may then need to transfer some stool into a stool cup. This part of the procedure can also be performed by the patient if they are able. In this case you need to provide the patient with detailed step by step instructions.

Use the stool cup spoon or a sterile wooden spatula, and scoop some of the stool into the smaller collection cup. Several big scoopfuls are needed, but no more than half a cup.

Throw away the stool leftover in the container or bag into the toilet

Leave the container in a designated location or throw away the bag in a designated biohazard bin

Ask the patient to hand over the filled collection cup. Make sure it is tightly sealed and correctly labelled at least with the patient's ID number.

Slide 15: To transfer stool onto a faecal swab.

Instead of stool in a stool cup, the laboratory may need a sample in the form of a swab in a tube of transport medium such as Cary Blair.

In this case, when you have the loose stool in a container, use a sterile cotton or polyester-tipped swab and dip it in the stool. Swirl or rotate it around for a few seconds. Be sure to pick up some mucus and shreds of tissue if any are present in the stool. Then transfer the swab tip first into the tube of transport medium, all the way to the bottom of the tube. Leave the swab in the tube, for this you may need to break off the tip. Close the tube and label it correctly.

You could also perform the RDT directly from the swab but in that case do not place it in the tube of Cary Blair then.

Slide 16: To collect a rectal swab.

In rare cases, you will need to perform a rectal swab. This is indeed rare with cholera patients as they tend to have profuse watery diarrhea.

Always ensure patient privacy and explain the procedure.

If two rectal swabs are required, collect them at the same time.

So to perform a rectal swab:

* Have the patient place themselves comfortably lying on their side with their top leg folded
* Open the wrapper of the swab by the handle end, and never touch the sterile tip of the swab
* Moisten the swab in sterile transport medium
* Insert the swab in the rectal sphincter about 3–4 cm
* Rotate the swab for 5 to 10 seconds, and withdraw gently
* Examine the swab and make sure there is visible fecal material. If not, repeat the procedure with a new swab.

Slide 17: How to handle a swab.

When you are working with sterile swabs, it is crucial to never touch the tip of the swab otherwise it is no longer sterile and you may contaminate the sample that you aim to collect.

Only open the new swab with clean gloves, and open it by the base of the swab and not the tip. When it is removed from its package, do not let the sterile tip touch anything else but the patient or the sample.

For some types of swabs. You may need to snap the swab handle so that it fits into the tube of Cary Blair.

Slide 18: Waste management.

As a general rule, any biological waste needs to be disposed of appropriately. This applies to any material that have been in contact with faecal matter.

If you are providing reusable buckets or containers, you will need to have a system in place to safely store, wash and sterilize these items before they can be used again. If you are providing disposable bags, there needs to be a biohazard bin available for the patient to throw it away and mechanisms in place to incinerate the biological waste and not let contaminated plastic disperse in the environment.

Slide 19: What next?

Now that you have either a stool sample in a collection cup or have a swab (a feacal swab or a rectal swab), what will you be doing next?

You may want to prepare the sample for transport to a laboratory. We will be discussing the procedures for this in the next part of this course.

You may also be required to directly test your sample using a rapid diagnostic test. We will discuss the procedures for testing with RDT in the next module.

Slide 20: SAMPLE STORAGE AND PREPARATION FOR TRANSPORT.

Sample storage and preparation for transport.

Slide 21: Preparing samples for transport.

There are different methods for preparing cholera samples for transport. The choice of the transport method will depend on:

* What resources are available. Do you have stool cups, Cary Blair swabs, etc
* How long will the sample take to get to the laboratory and when it is expected to be tested. Will it get to be tested in less than 2 hours or more than 2 hours.
* And what type of tests are to be performed at the laboratory. Will the lab be culturing the sample, or doing PCR for example.

These decisions are usually made by the laboratory in coordination with the surveillance team. Make sure you are always aware of the procedures required.

Slide 22: Preparing samples for transport.

There are 5 most common ways to prepare samples for shipping to the laboratory:

* A sample can be sent directly in the stool cup
* A sample can be sent in as a swab in Cary Blair transport media
* A sample can be sent in a tube of Alkaline Peptone Water
* A sample can be sent on wet filter paper
* A sample can be sent on dry filter paper

We will go through the procedures and characteristics of all these methods.

Slide 23: Safely collect samples.

Before starting the procedure, the basic safety and hygiene practices. These are key to keeping you and your community safe.

Wear gloves every time you collect or handle a stool sample.

If you have any cuts or abrasions on your skin, if possible cover them with adhesive dressing or skin tape first.

When you are done collecting and handling the sample, remove the gloves and wash

your hands with soap.

Use a new pair of gloves and wash your hands in between each patient. You may also protect your clothes by wearing scrubs or a lab coat.

Be sure to adhere to proper procedures for the disposal of any waste.

Slide 24: SAMPLE In a Cary Blair Swab.

The most favored transport medium for specimens with suspected Vibrio cholerae is a medium called Cary Blair

You will need tubes with sterile Cary Blair medium in them. In general these tubes come with sterile swabs

If your initial sample is loose stool in a container, use a sterile swab to dip in the stool, swirl it about and transfer the swab all the way down into the tube of Cary Blair and leave it in the tube.

If you had to perform a rectal swab, you can place the rectal swab directly into the tube of Cary Blair.

On average, these samples can be stored and transported for 7 days at ambient

temperature. Be careful to keep any samples out of direct sunlight.

The laboratory will be able to perform culture directly from these samples, or even RDT and molecular testing but only after further processing.

Slide 25: Sample in a Stool cup.

Stool samples can be sent to the laboratory in the initial simple stool cup but they would need to be tested rapidly within 2 hours of collection.

These samples must be sent at ambient temperature, ideally between 22 and 25 degrees centigrade. There is generally no need to add icepacks unless the temperature is expected to go above 35 degrees.

From these types of samples, if the tests are done rapidly, the laboratory can perform a RDT, culture or even molecular analysis.

Slide 26: Sample in Alkaline Peptone Water (APW).

A sample can be suspended in Alkaline Peptone Water to favor the growth of Vibrio cholerae. This is generally not the preferred method as it requires more preparation and must be received quickly at the laboratory.

You will need prepared tubes of sterile APW and transfer pipettes or swabs.

Using a transfer pipette or swab, transfer some stool from the initial container into a tube of APW in a way that the stool should not take up more than 10% of the total volume of APW.

These samples must be tested within less than 24 hours.

They should be transported at ambient temperature

And finally, this preparation in APW can be used in the laboratory for RDT, culture and molecular testing.

Slide 27: SAMPLE ON Wet Filter paper.

A sample can be sent on wet filter paper.

You will require filter paper disks, some saline solution, a small tube with a screw cap and a single-use needle or forceps.

Use the single-use needle or forceps to dip the disk in the stool, then place that disk in the small tube, add 2 or 3 drops of saline solution and close the tube.

This way, the sample can be stored or transported for a maximum of 15 days at ambient temperature.

From these samples, the laboratory can perform culture or molecular tests but they will need extra processing before an RDT can be performed.

Slide 28: SAMPLE ON DRY FILTER PAPER.

Samples can also be sent on dry filter paper.

For this method you will need whatman cards, disposable transfer pipettes, individual plastic pouches and a desiccant.

Use the transfer pipette to deposit one drop of watery stool onto the filter paper. Let the paper air dry before placing it into an individual pouch with desiccant.

Using this method, a sample can theoretically be kept indefinitely in ambient temperature however the laboratory will only be able to perform molecular analysis such as PCR and not culture or RDT.

Slide 29: Labelling specimens for transport.

Accurately labeling samples is critical.

At a minimum, each specimen should be identified by:

* patient’s name and/or unique identification number
* The time and date of collection of the sample
* The initials or name of the person having collected the sample

Other information can be important such as what test has been ordered.

If possible, use computer-generated bar codes linked to the complete electronic information of the patient.

Be sure to adhere to local guidelines within the country you are in.

Slide 30: SAMPLE TRANSPORT.

Sample transport

Slide 31: Sample transport.

When we speak of sample transport, we aim to move a sample from one location, usually a health facility but sometimes a laboratory, to another location for instance a laboratory. We want to do this in a way that is safe – so that neither the person transporting the sample, nor the community or the environment be exposed to what is in the sample -, that is secure from theft for example, and in a way that preserves the quality of the sample so that it can be further analyzed.

Slide 32: Requirements.

There should be readily available procedures for sampling, packaging, and transport. These procedures should take into consideration the conditions of the transport system: how are the samples going to be transported for example, by motorbike, by car, by plane? how long will the samples need to get to the laboratory? There may be different procedures for different transport mechanisms.

You should also have readily available sample referral or transfer forms to share the patient information along with the samples in a standardized way.

Slide 33: Transport regulations.

It is critical to follow all applicable regulations when transporting infectious agents.

Regulations for the transport of biological samples an infectious agents come from several international and national organizations to ensure safety, security, and compliance. The key sources include:

The International Air Transport Association (IATA), the International Civil Aviation Organization (ICAO), rail, road and sea traffic agencies, postal services and even private couriers.

You can find training and certifying courses available online.

Slide 34: Triple packaging.

To transport samples, we use what is called triple packaging.

First each specimen should be protected by a primary container. This is a leak-proof, sterile and labeled container and generally it is a stool cup, or a tube with a swab in Cary Blair for example.

Surrounding the primary container, there should be a layer of absorbent material such as tissue paper. There needs to be enough absorbent material to absorb all the content of the primary container if it leaks.

A secondary container is then used, such as a plastic can with a lid and this is sealed to contain further leakage.

Finally, a tertiary container such as a cardboard box or a cooler is used to protect all samples from physical damages or external pressure.

Triple packaging should be used whether a sample is transported locally in country, or internationally.

Slide 35: Domestic transport.

In this example, you’ll see an illustration of how the example materials are packaged together for transport in country.

Slide 36: International Transport.

For international transport of samples, specific packaging and materials may be required. There are further certifying courses available to individuals responsible for international shipments.

Slide 37: Transport DOS and DON’TS.

Cold temperatures decrease the viability of *Vibrio cholerae* in the stool.

So for the best quality stool samples for cholera testing, the samples should be transported at ambient temperature or temperatures ranging between 22 and 25 degrees.

We do not want to use ice packs unless the temperature is expected to go beyond 35 degrees.

If ice packs are a must, then they should be placed between the secondary and tertiary containers and not directly in contact with the samples.

Do not leave boxes exposed to direct sun for prolonged periods.

Again, in most situations, ice packs are really not needed and could damage the samples.

Slide 38: REFERRAL FORMS.

Referral forms

Slide 39: Few considerations!

Communication between field staff and laboratory staff is critical to ensure that good quality specimens are submitted for testing.

It is important for the field staff to send complete and accurate information about the patient together with the sample.

If there are any questions or missing information, it will be important for the

laboratory staff to communicate with the field staff to find the answers. The field staff may also need precious advice from the laboratory about the procedures to use for sample collection and preparation.

Communication should go both ways.

Slide 40: GTFCC Laboratory referral form for cholera suspected case.

Here is an example of a standardized referral form that is filled and accompanies every single sample that is sent to the laboratory. This one was developed by the GTFCC and can be adapted to your needs.

Slide 41: Minimum recommendations for referral.

Sample referral forms hold key information about the patient and the sample. They should include at the very least the following information:

A unique patient identifier and, if used, a unique sample identifier

The name of the patient, age, sex and address

The name of the referring health facility or person

The date of specimen collection

The result of the rapid diagnostic test if one was performed

Slide 42: Sample referral forms are critical.

The information contained in these forms is critical.

Without any of this information, the laboratory may struggle to perform the correct test, or results will be reported for the wrong patients.

Similarly, if the information provided is incomplete, the laboratory will have difficulties matching the results to the correct patients or maybe will not be able to draw full conclusion.

In either case, this will lead to an inaccurate picture of the outbreak.

In both cases the laboratory would probably reject the sample.

Hence communication is key. If you have incomplete information, try to complete the gaps and if it’s not possible, let the laboratory know. Supplying the laboratory with the correct information, using the right forms, clearly labeling the sample and placing the documents and sample referral forms with the sample itself as we mentioned before, is crucial for a successful operation.

Slide 43: Referral and reporting.

Here are a few additional tips.

Always write and double check the patient ID or sample ID.

Use standardized sample referral forms and provide these forms to all health facilities collecting samples.

Use one form for each sample collected from any one single patient.

Complete each form with as much information as possible or specify the instances where the information is not available.

Place the forms and other documents with the samples in an impermeable sleeve, in a way where there will not be dirtied by a leaking sample.

If possible, send an electronic copy or the forms to the laboratory at the same time and keep a copy for your facility.

Inform the laboratory that a sample is on its way.

Slide 44: Links to GTFCC support material.

Here are links to GTFCC documents that further detail everything discussed during this module. Do not hesitate to take a look and print out what you need.

Slide 45-47: END OF MODULE ASSESSMENT.