



GLOBAL TASK FORCE ON
CHOLERA CONTROL

GTFFCC recommendations

SAMPLE COLLECTION, PREPARATION AND TRANSPORT FOR CHOLERA

V1.0 November 2024





Learning objectives

- Describe what is a good specimen for cholera testing

- Safely collect and prepare good quality samples for cholera testing

- Define the key factors affecting storage and transport of samples

- Identify what is needed for adequate transport of samples

- Apply best practices in reporting

Suggested previous modules: module 1 Introduction to Cholera and Cholera testing

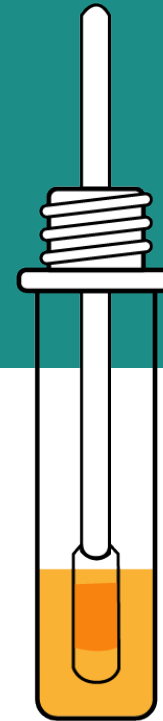


Outline

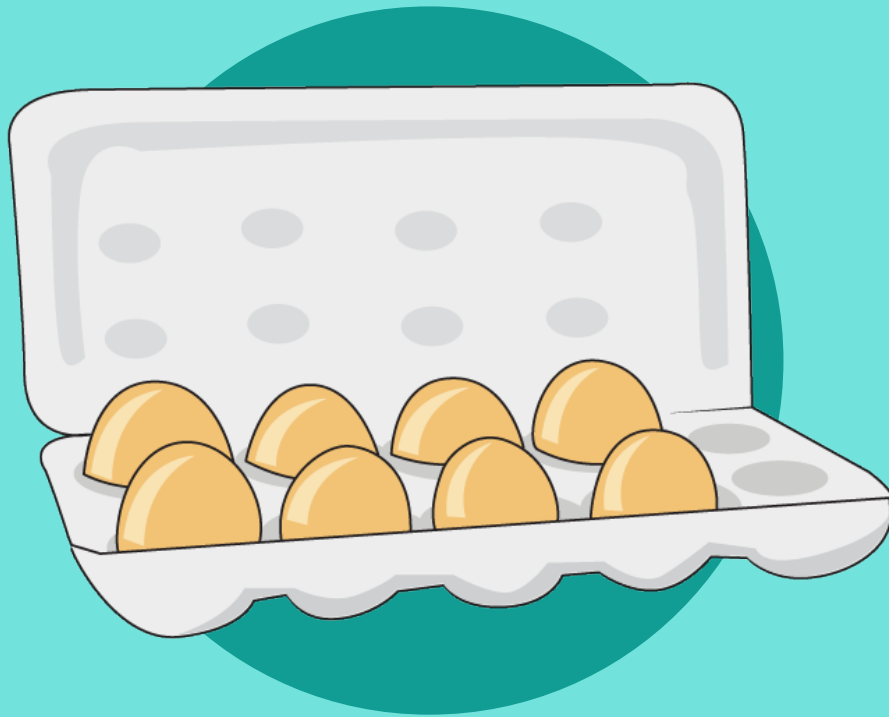
- 1 Sample collection
- 2 Sample storage and preparation for transport
- 3 Sample transport
- 4 Referral forms
- 5 End of module assessment



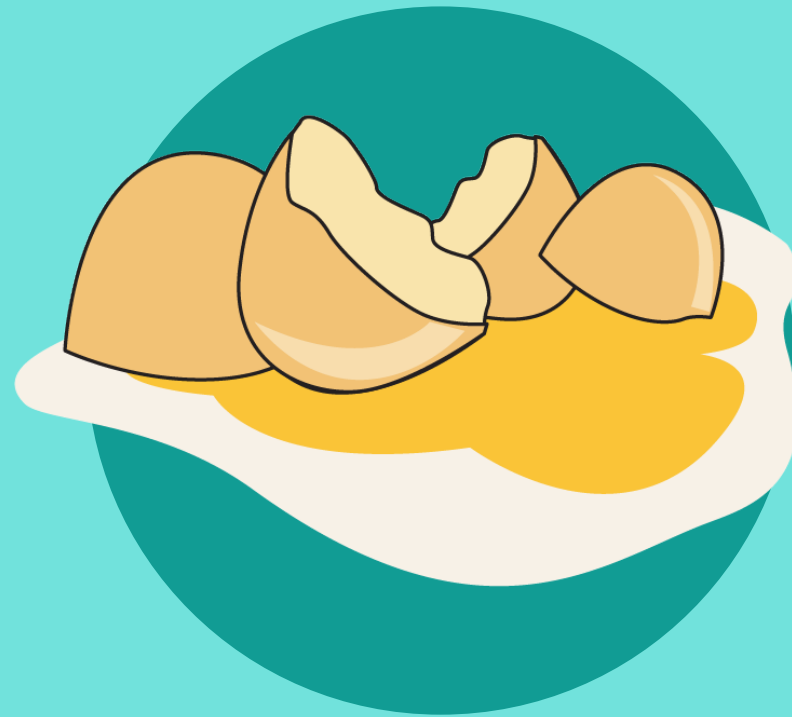
SAMPLE COLLECTION



“ *The result of any laboratory examination is only as good as the sample received in the laboratory.* ”



Good sample



Bad sample



Outcomes of sample collection

The first step to perform any type of testing for cholera is adequate sample collection

Outcomes of **adequate** specimen collection and transport :

- Accurate, reliable test results
- Correct diagnosis and effective treatment
- On-time reporting of test results
- High customer satisfaction
- Reduced costs

Outcomes of **inadequate** specimen collection and transport :

- Questionable test results
- Incorrect diagnosis and ineffective treatment in case of antibiotic need
- Delays in reporting of test results
- Unnecessary re-testing
- Decreased customer satisfaction
- Increased costs
- Injury
- Death

Specimen criteria for cholera testing

Stool or rectal swabs are the recommended specimen types for cholera testing.

Specimens should be collected from patients who:

Fit the definition for a suspected case of cholera within the testing strategy

Refer to module 1:
Introduction to Cholera
and Cholera testing

Preferably, are within 4 days of onset of symptoms (when the bacteria are found in greater numbers in the stool)

Preferably, have not taken antibiotics

Antibiotics

Patient care should always be prioritized. Specimens from patients who have not taken antibiotics are preferred as antibiotics may reduce the number of bacteria in the sample hence impacting test accuracy.

If the patient has taken antibiotics, report this information in the sample collection forms:

- ▶ Which antibiotic was taken?
- ▶ How much / dosage?
- ▶ When / for how long?





Basic hygiene practices

Safely collect samples

Protect yourself, your patients and your community.



If you have cuts or abrasions on the skin of your hands, cover them with adhesive dressing.



Wear gloves when collecting and handling stool specimens.



Remove gloves and wash your hands after completing any task involving the handling of stool specimens.



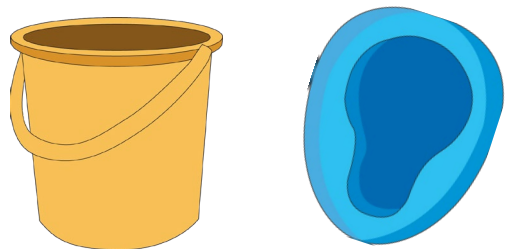
Adhere to proper waste disposal procedures.

You can also protect your clothes by wearing scrubs or a lab coat.

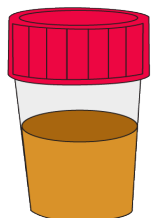
How to collect specimens for cholera testing

Able and bed-ridden patients
who **produce** loose stool

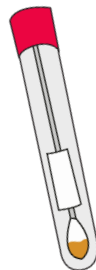
Primary container



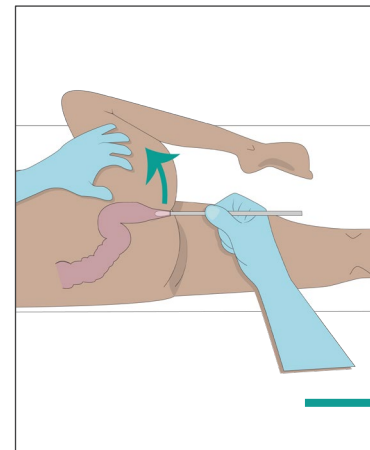
Stool cup



Fecal swab



Patients who
do not produce loose stool



Rectal swab



After further sample preparation, you may have other types of samples such as a sample on filter paper or in Alkaline Peptone Water as presented in the next section.

<https://www.gtfcc.org/resources/specimen-collection-preparation-and-packaging-for-transport/>

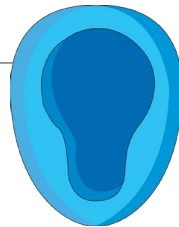


All about bleach

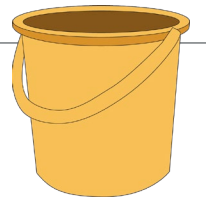
Bleach and other disinfectants can kill the bacterium and lead to incorrect test results.



Always collect stool samples from patients using clean containers - without *disinfectant or detergent residue or other contaminants (urine etc.)*.



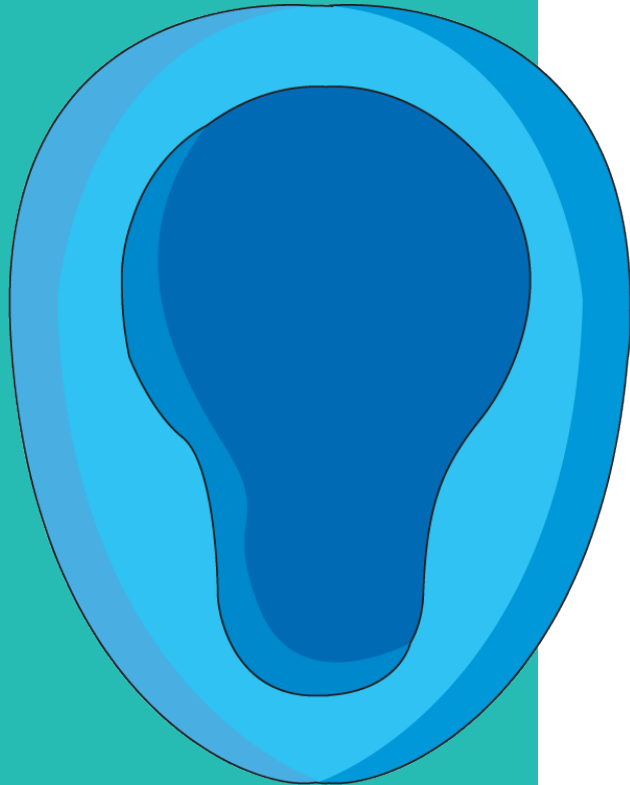
Before use, bedpans and buckets should be washed, disinfected, rinsed thoroughly with clean water and dried.





Able patients

- ✓ Provide the patient with a container such as a bucket/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.
- ✓ Instruct the patient to:
 - 1) urinate before using the container
 - 2) pass stool in the container



Bed-ridden patients

- ✓ Use a clean, unused bedpan or bucket. A bedpan or bucket must be washed, bleached, thoroughly rinsed and well dried before being reused.
- ✓ The bedpan or bucket must not have any residue of chlorine or any other disinfectant.
- ✓ Place it under the hole of a cholera bed or under the patient.
- ✓ Collect freshly passed stool.

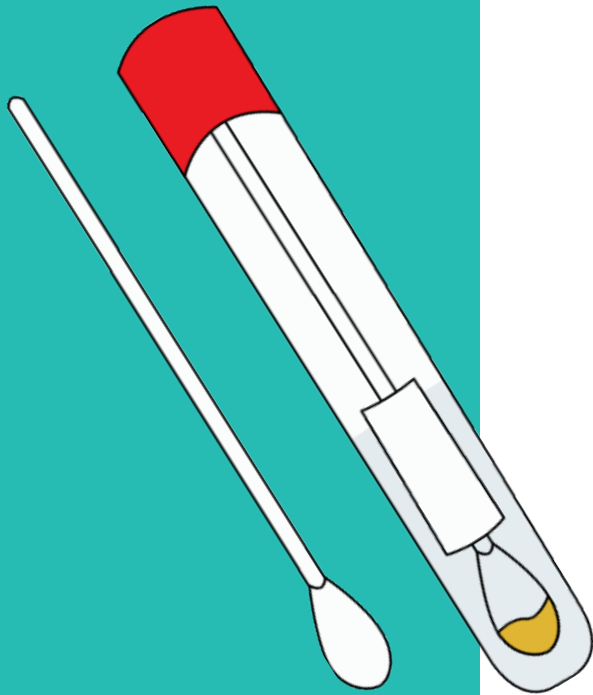


To transfer stool to a stool cup

This part of the procedure may be performed by the staff or patient if adequately instructed:

- ✓ Transfer stool from the initial container into a stool cup using the provided spoon, a sterile spatula, or a swab. Fill the stool cup no more than halfway.
- ✓ Empty the leftover stool from the bucket/plastic bag into the toilet, latrine or sewage pit.
- ✓ Leave the dirty bucket/plastic bag in a designated location or discard the plastic bag in the appropriate biohazard bin.
- ✓ Remove gloves and wash hands.
- ✓ Return the filled stool cup to health worker in charge.

Make sure the lid of the stool cup is tightly closed and that it is correctly labelled!

PROCEDURE FOR
FAECAL SWAB

To transfer stool onto a faecal swab

- ✓ Use a sterile cotton or polyester-tipped swab.
- ✓ Dip swab and swirl it in the stool.
- ✓ Use the swab to sample any mucus or tissue shreds, if present.
- ✓ For RDT: do not place swab in transport medium and continue immediately with the RDT.
- ✓ For transport: place the swab tip first all the way in the tube of transport medium (most often Cary Blair medium).

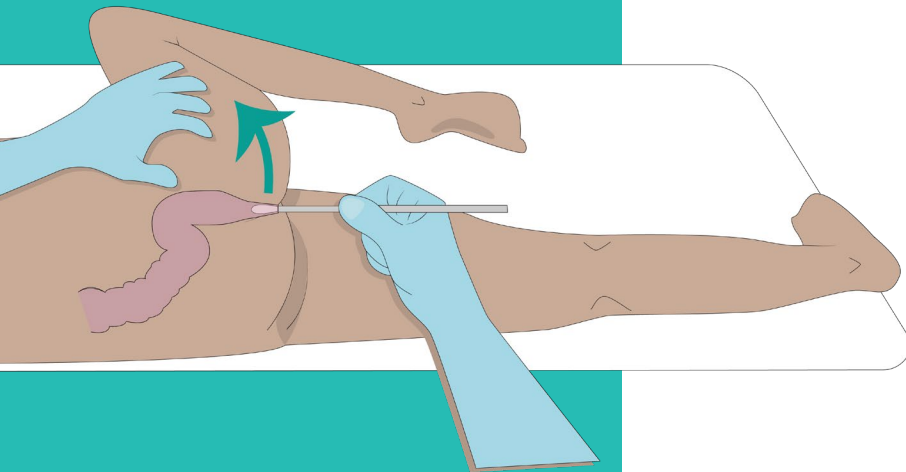
Make sure the tube is tightly closed and correctly labelled!

To collect a rectal swab

Ensure patient privacy and explain the procedure.

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

- 1 Ask the patient to lie on their side and to bend and lift their knee that is most on top.
- 2 Moisten the swab with the sterile transport medium or with saline provided.
- 3 Insert the swab through the rectal sphincter 3-4 cm.
- 4 Rotate for 5 to 10 seconds, and withdraw with care.
- 5 Examine to ensure there is faecal material visible on the swab.



How to handle a swab

Always unwrap a sterile swab without touching the tip and without letting the tip touch anything else to avoid contamination.



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





Waste management

Any biological waste needs to be disposed of appropriately, this applies to any materials that have been in contact with faecal matter.


If you are providing patients with reusable buckets or containers to collect stool, 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 patients with disposable bags, there needs to be a biohazard bin available for the patient to throw the bag away and mechanisms in place to incinerate the biological waste and not let contaminated plastic disperse in the environment.




What next?


You now have a stool sample in a collection cup or have a swab (faecal swab or a rectal swab)



Prepare the sample for transport to a laboratory



Directly test using a Rapid Diagnostic Test (RDT)*

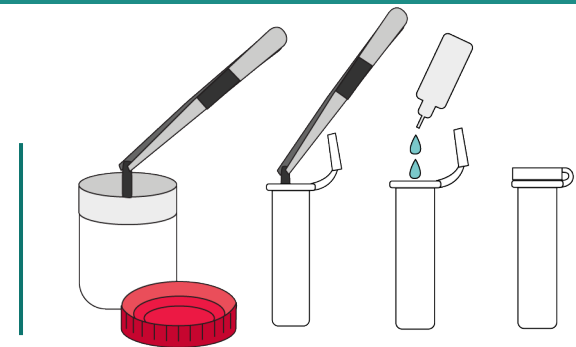


Go to module 3:
Cholera Rapid Diagnostic tests

**Not all types of samples can be used for a direct RDT*



SAMPLE STORAGE AND PREPARATION FOR TRANSPORT





Preparing samples for transport

There are different methods of preparing cholera samples for transport.

The method used will depend on:

1

What resources are available (stool cups, Cary Blair swabs, etc.)

2

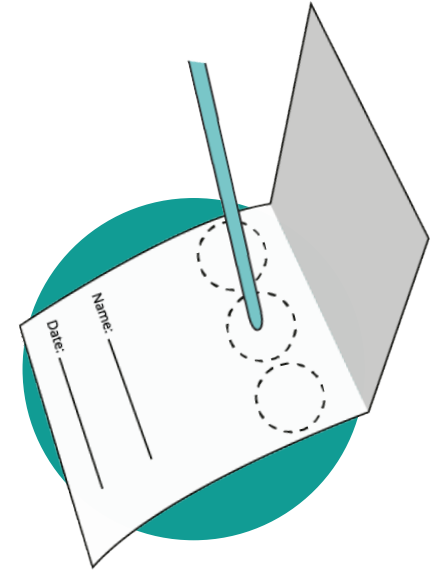
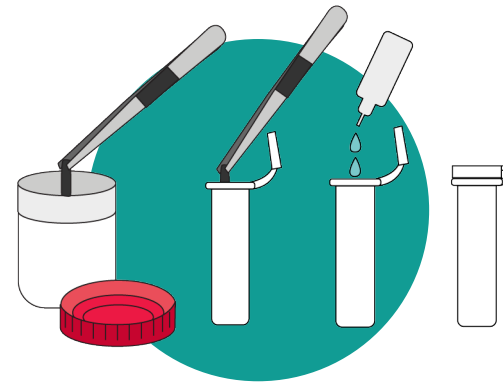
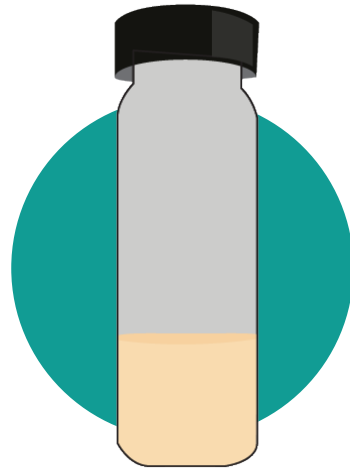
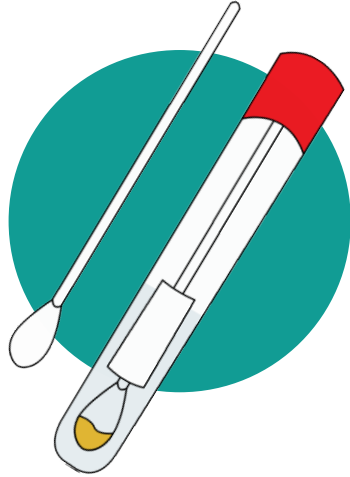
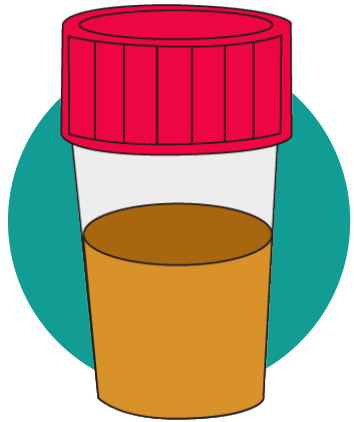
When the sample is expected to be tested (within 2 hours, >2 hours...)

3

What type of tests are to be performed (RDT, culture, PCR etc..)

These decisions are to be made in advance by the cholera laboratory in coordination with the surveillance team!

Preparing samples for transport



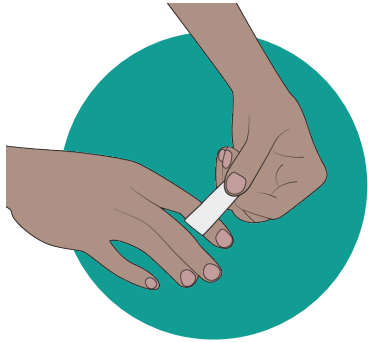
5 most commonly recommended ways to prepare samples



Basic hygiene practices

Safely collect samples

Protect yourself, your patients and your community.



If you have cuts or abrasions on the skin of your hands, cover them with adhesive dressing.



Wear gloves when collecting and handling stool specimens.



Remove gloves and wash your hands after completing any task involving the handling of stool specimens.



Adhere to proper waste disposal procedures.

You can also protect your clothes by wearing scrubs or a lab coat.

SAMPLE IN A CARY BLAIR SWAB

*The most favored transport medium for suspected *V. cholerae* specimens*

Material Required

Cary Blair
semi-solid,
bottle/tube

Swab
sterile,
cotton/polyester

Compatibility with
testing methods*

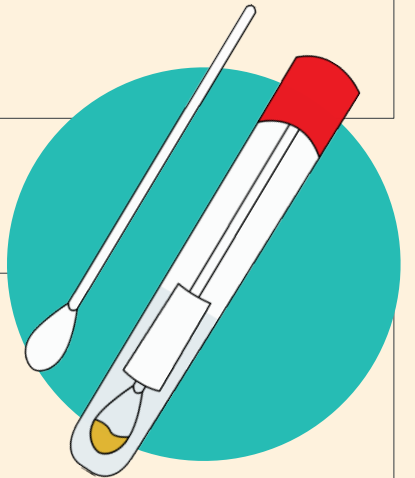
Culture

Conservation

- For testing within an average of 7 days from collection.
- Should be transported at ambient temperatures (22 °C - 25 °C).
- Keep container out of direct sunlight.

Procedure

- **For faecal samples:** dip swab in stool and transfer into Cary Blair tube.
- **Rectal swab:** place swab directly into Cary Blair tube. No further manipulation is needed.



SAMPLE IN A STOOL CUP

Material Required

Stool container
plastic, screw on
cap, 30ml, without
disinfectant

Compatibility with testing methods

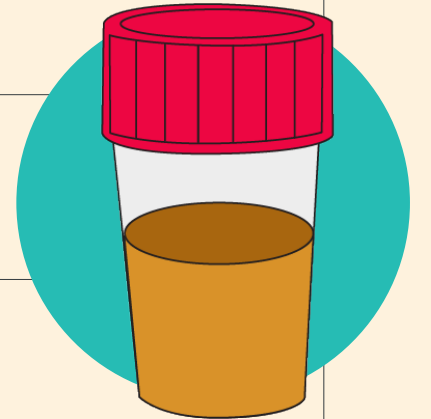
RDT
Culture
PCR...

Conservation

- For testing within 2 hours from collection only.
- Should be transported at ambient temperatures (22 °C - 25 °C).
- Keep container out of direct sunlight.

Procedure

- Keep in initial stool cup.
- Parafilm or sealing tape can be used to seal container and prevent leakage when possible!



SAMPLE IN ALKALINE PEPTONE WATER (APW)

Material Required

APW

tubes with screw cap, transfer pipettes or swabs

Compatibility with testing methods

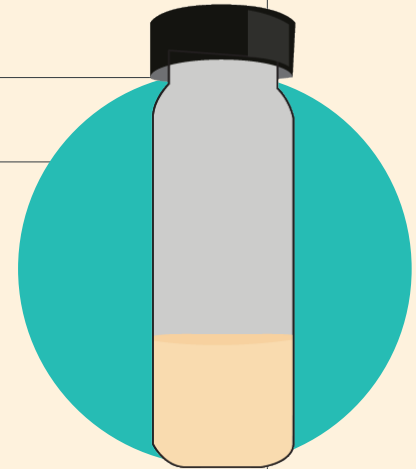
RDT
Culture
PCR...

Conservation

- For testing within 24 hours from collection only.
- Should be transported at ambient temperatures (22 °C - 25 °C).
- Keep container out of direct sunlight.

Procedure

- Transfer faecal matter from initial container into APW tube.
- Parafilm or sealing tape to seal container and prevent leakage when possible!
- **NOTE:** the faecal matter should not exceed 10% of the volume of the APW enrichment.



SAMPLE ON WET FILTER PAPER

Material Required

WFP

Filter paper discs
(6mm Ø, non-sterile),
sterile saline solution,
forceps
or needle,
2ml tube (screw cap)

Compatibility with testing methods

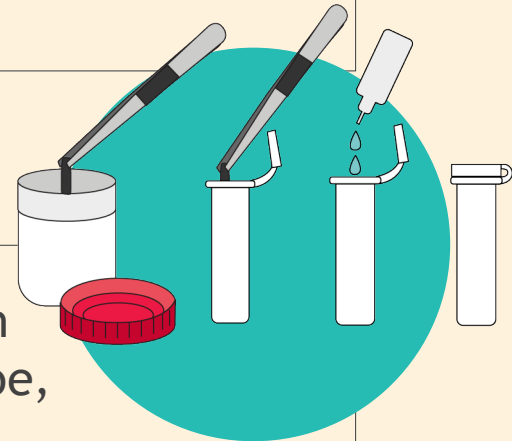
RD^T*
Culture
PCR...

Conservation

- For testing within 15 days from collection.
- Should be transported at ambient temperatures (22 °C - 25 °C).
- Keep container out of direct sunlight.

Procedure

- Dip filter paper disk into watery faecal material with single-use device (forceps, needle), transfer into tube, add 2 to 3 drops of sterile saline, close tube.



*RD^T requires an enrichment step in APW

SAMPLE ON DRY FILTER PAPER

Material Required

DFP

Whatman cards (903 protein saver, FTA Elute Micro Cards), disposable transfer pipettes, individual pouches, desiccant

Compatibility with testing methods*

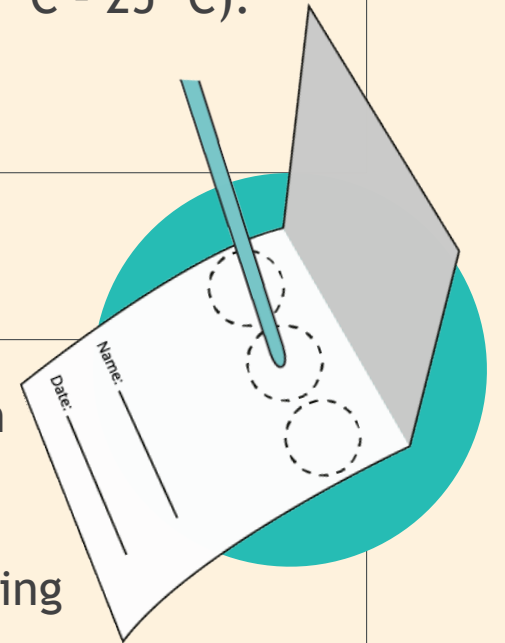
PCR...

Conservation

- No limitation to duration of conservation.
- Should be transported at ambient temperatures (22 °C - 25 °C).
- Keep container out of direct sunlight.

Procedure

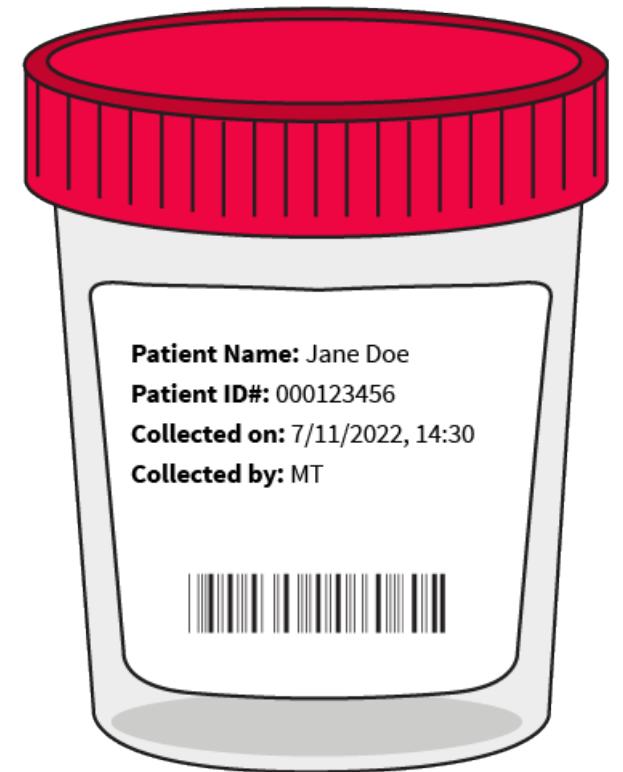
- Deposit one drop of watery stool filling the circle on filter paper.
- Air dry paper in a clean, dust free area, before placing into individual pouch with desiccant.



**Used for molecular testing only • Cannot be used for culture or RDT*

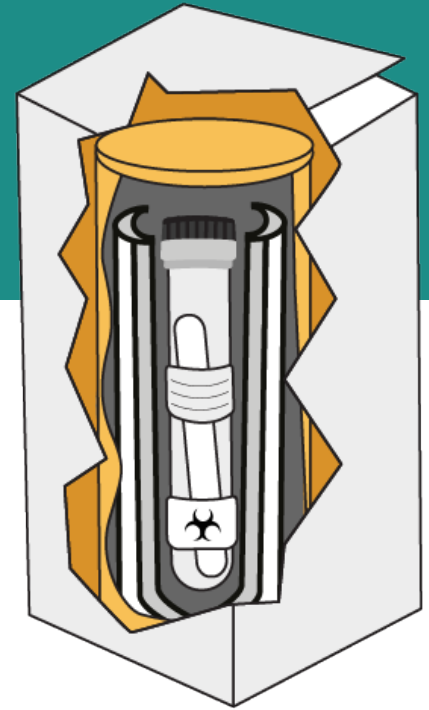
Labelling specimens for transport

- As a minimum, each specimen should be labelled with:
 - Patient name
 - Unique identification number
 - Time and date of collection
 - Collector's initials
- Use computer-generated barcodes if possible.
- Be sure to adhere to the national procedures in place.





SAMPLE TRANSPORT





Sample transport

Objective: move a sample from one location to another in a way that is :



Safe

for the person, the
community and the
environment



Secure



Preserves
the quality of
the sample



Requirements



Readily available
procedures for
sampling, packaging,
and transport



Procedures that take
into account the
conditions of transport
(motorbike, car,
duration, etc...)



Readily available
sample transfer form to
be shared with the
samples

Moving any sample from any location to another, domestic or international, must comply with all packaging recommendations



Transport regulations

Where do they come from? Who makes them?

National and international transport regulations

ICAO/IATA transport regulations (air)

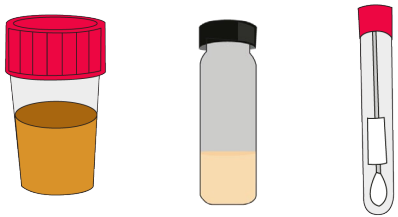
Rail, road, and sea traffic agencies

Postal services

Private couriers

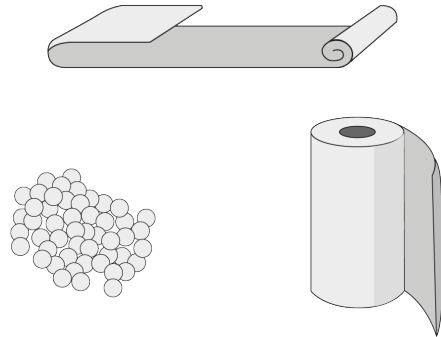
Triple packaging

Each specimen needs to be protected by



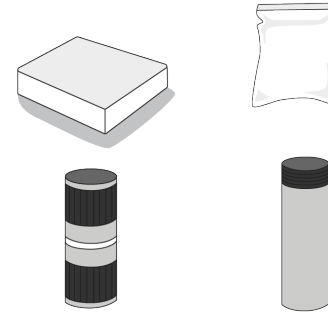
Primary container
(stool cup, Cary Blair
tube etc.) = material
that contains the
sample

Leak-proof, sterile,
labeled



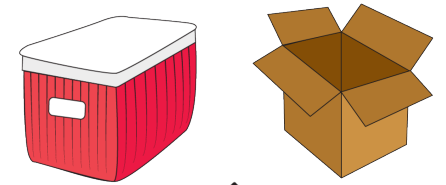
Surrounded by
absorbent material

To absorb potential
leakage



Secondary container
(plastic bag, canister
etc.)

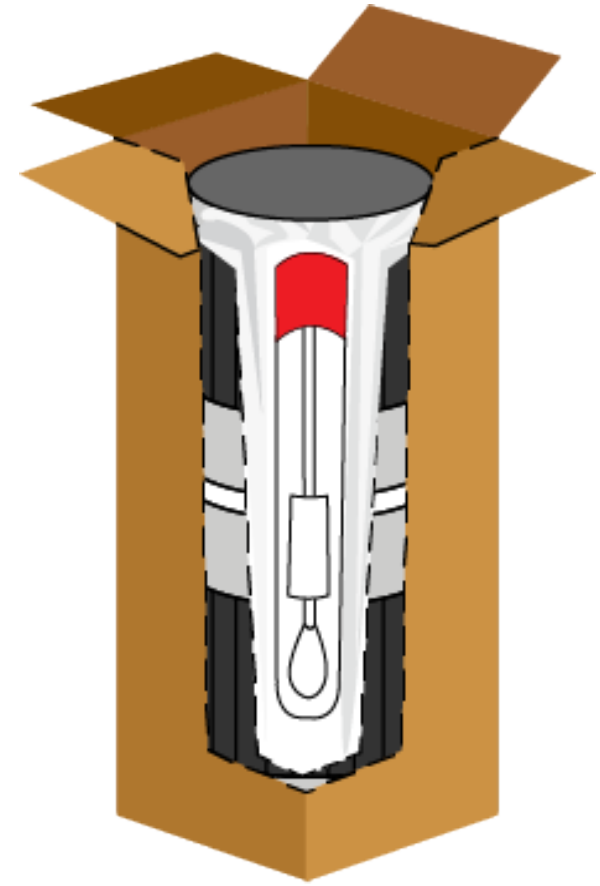
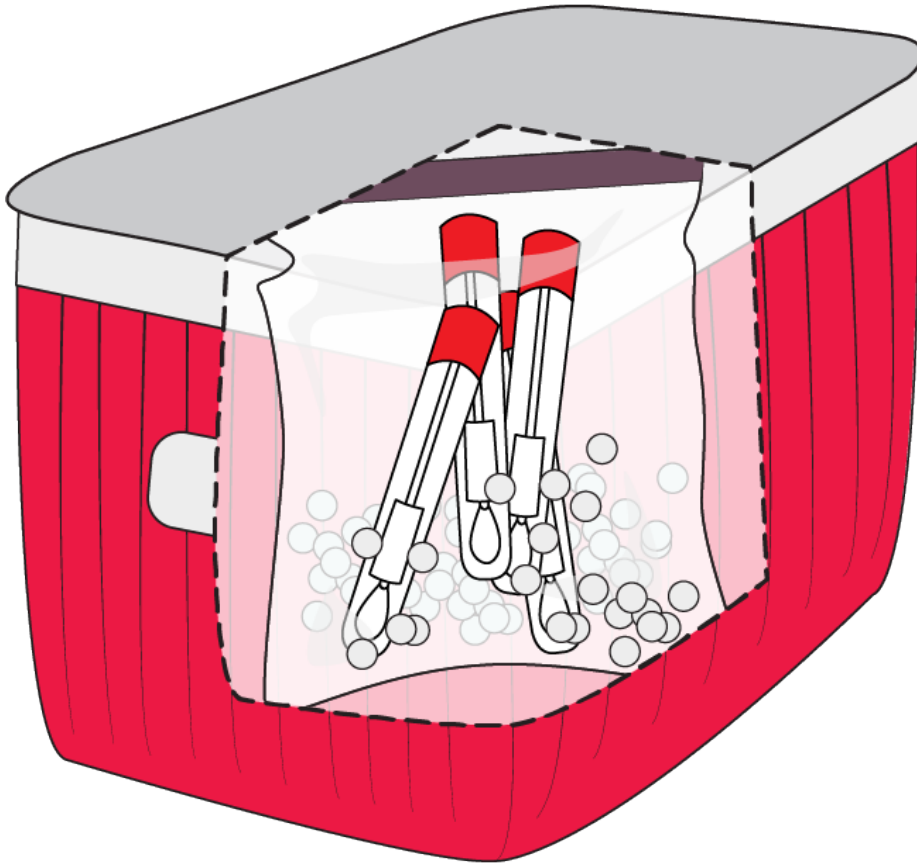
To contain leakage
and physical
protection



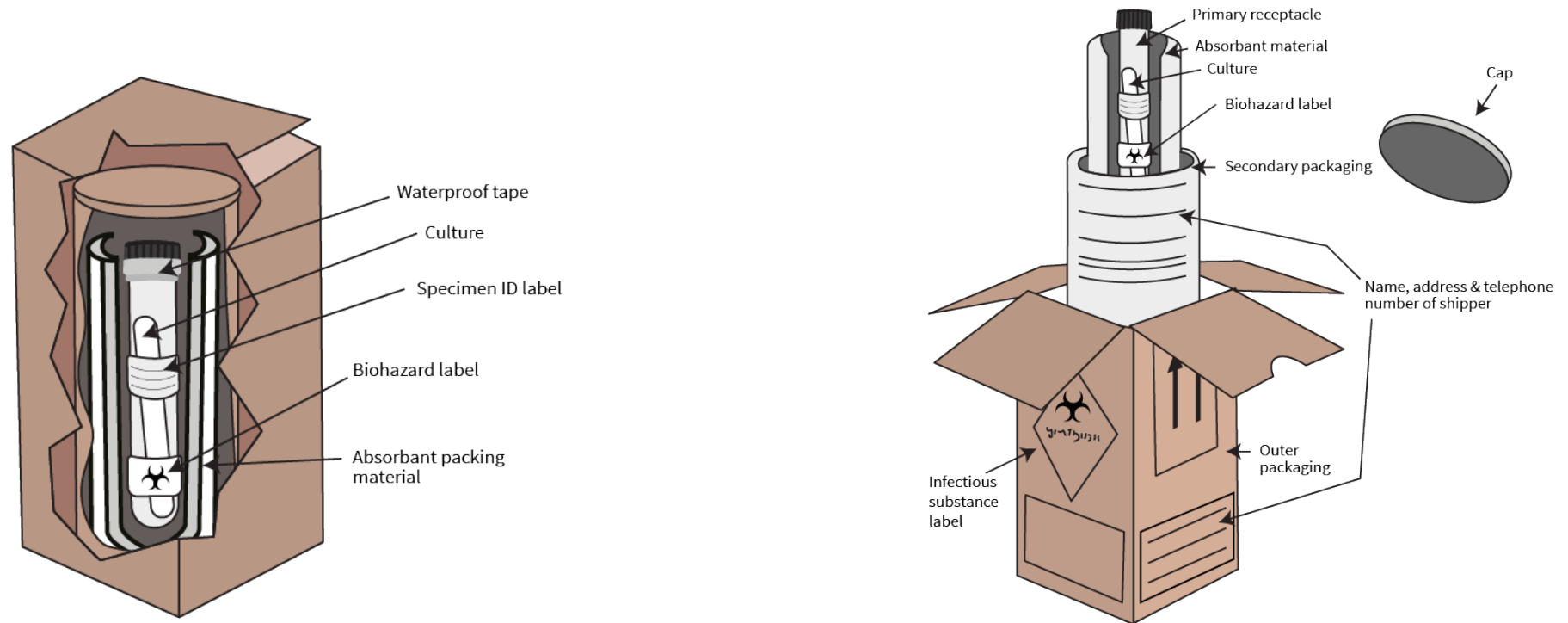
Tertiary container
(cooler or box etc.)

Physical protection
against damage and
external pressure


Domestic transport



International Transport



- International transport must abide by international regulations (IATA).
- Specific, labelled packaging may be required.



Transport DOS AND DON'TS

For the best quality of stool samples for cholera testing, stool should be kept at ambient temperature (22°C - 25°C).

If using ice packs, make sure to place them between secondary and tertiary containers and not in direct contact with the stool samples.

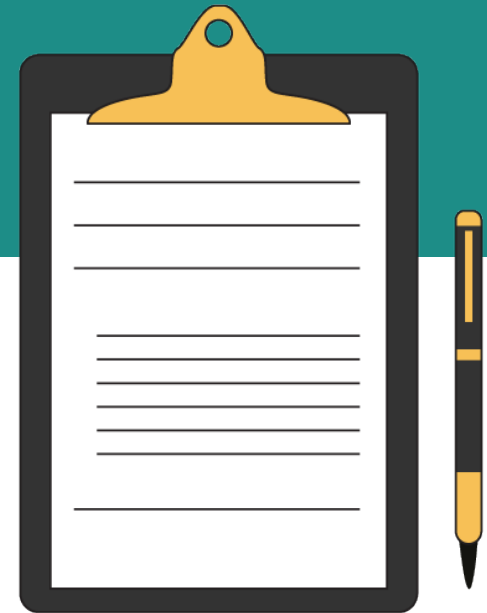
In most situations, ice packs are not needed.

Do not add ice packs unless temperature is expected to go above 35°C.

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



REFERRAL FORMS



Few considerations!




Communication
should go
both ways



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

GTFCC Laboratory referral form for cholera suspected case

 GLOBAL TASK FORCE ON
CHOLERA CONTROL

GTFCC Laboratory Referral Form for Cholera Suspected Case

The referring health worker is to complete this form and send a copy to the laboratory with the specimen (one form per specimen sent).
Please attach a copy of the Admission and Triage Form.
For specific instructions for packaging and transportation please refer to Specimen Packaging and Domestic Transportation for Laboratory Confirmation of *Vibrio cholerae* O1/O139.

Request made by _____

Name of health facility (or stamp or health facility identifier)

Date of request: ____/____/____
Name of referring health worker: _____
Address: _____
Phone: _____ E-mail: _____

Request made for _____

☐ Laboratory identification of Cholera ☐ Antimicrobial Susceptibility Testing ☐ Other, specify: _____

Specimen _____

Specimen ID: _____ Date and time of collection: ____/____/____ ____:____:____

Location specimen collected: _____

Type of specimen collected: ☐ Stool ☐ Rectal swab ☐ Other, specify: _____

Blood observed in stool: ☐ Yes ☐ No

Appearance of specimen: ☐ Formed ☐ Soft ☐ Watery ☐ Bloody-mucus

Conditioning of stool sample: ☐ Stool in container (no added reagents) ☐ In Cary Blair ☐ In Alkaline Peptone Water (APW)
☐ on filter paper ☐ other, specify: _____

Date specimen sent to referral laboratory: ____/____/____

If date of specimen collection and date specimen sent are different, how was the specimen stored (media, temperature)? _____

Was an RDT performed on the same specimen? ☐ No ☐ Yes, specify: ☐ Enriched RDT ☐ Direct RDT
Result: ☐ Reactive O1 ☐ Reactive O139 ☐ Reactive O1 and O139 ☐ Non-reactive ☐ Invalid
Name of RDT kit used: _____

1 To be sent at ambient temperature (ideally 22-25°C). Do not refrigerate or freeze. Keep out of sunlight.

Page 1 of 2 / Specimen ID: _____

Patient _____

Last name: _____ First name: _____

Patient ID: _____ Sex: ☐ Male ☐ Female

Age: ____ Years / ____ Months / ____ Days or Date of birth ____/____/____
____/____/____

Date of onset of illness: ____/____/____

Where did the patient first feel sick? Region/Province _____ District _____ Town _____

Patient outcome at time of request: ☐ Hospitalized ☐ Discharged ☐ Deceased ☐ Self-discharged
☐ Referred, specify _____ ☐ Unknown

Antibiotics treatment received prior to collection of sample: ☐ No ☐ Yes
**Any antibiotics received by the patient prior to sample collection may negatively impact laboratory results.*
Specify which antibiotic: _____
Specify dose of antibiotic: _____ Specify duration of treatment (days): ____/____/____
____/____/____

Oral Cholera Vaccine (OCV) received: ☐ Unknown ☐ No ☐ Yes When? ____/____/____

Name of OCV: _____

Relevant travel history _____

Signs & symptoms

<input type="checkbox"/> Watery stool _____ days	<input type="checkbox"/> Respiratory distress
<input type="checkbox"/> Bloody stool _____ days	<input type="checkbox"/> Sunken eyes
<input type="checkbox"/> Vomiting _____ days	<input type="checkbox"/> Unable to drink or drinking poorly
<input type="checkbox"/> Fever _____ days	<input type="checkbox"/> Skin pinch going back very slowly (>2 seconds)
<input type="checkbox"/> Lethargy	Other symptoms _____
<input type="checkbox"/> Loss of consciousness	Any known contacts with anyone with similar symptoms?
<input type="checkbox"/> Absent or weak pulse	<input type="checkbox"/> No <input type="checkbox"/> Yes, specify _____

To be completed upon reception of the specimen by the receiving laboratory _____

Recipient laboratory (name/ address or stamp)

Name of lab personnel performing sample intake: _____

Date and time of specimen received: ____/____/____ ____:____:____

Condition of specimen/packaging/documentation: ☐ Adequate ☐ Not adequate

If not adequate, specify (e.g. leaking, missing information, inadequate transportation or conservation temperature): _____

Follow up actions: ☐ obtain a second sample ☐ complete missing information ☐ other, specify: _____

Page 2 of 2 / Specimen ID: _____

<https://www.gtfcc.org/resources/gtfcc-laboratory-referral-and-results-reporting-forms/>



Minimum recommendations for referral

Unique patient identifier

Full name, age, gender at birth, address

Name of the referring health facility

Date of specimen collection

Result of RDT (if it was performed)

Sample referral forms are critical

No information at all

Problems in performing the correct test, problems matching results to a patient, surveillance teams get wrong results.

Missing/incomplete information

Problems in matching results to patients, surveillance teams misinformed, laboratory cannot draw a full conclusion.

Inaccurate picture of the outbreak!

Communication
is key



Referral and reporting

- Always write and check the patient ID/sample ID so the results can be matched to a patient.
- Use standardized sample referral forms.
- Use one form for samples collected on one patient at one time.
- Complete each form with as much information as possible - Saying you have no information is also information!
- Make sure that all the forms and documents are placed in an impermeable sleeve with the samples in a way they would not become soiled with the samples in case of any leaks.
- If possible, send an electronic copy of the forms to the lab and keep a copy on hand.
- Inform the lab that a sample is on the way!



Links to GTFCC support material

Recommendations for “Public Health Surveillance for Cholera” :

<https://www.gtfcc.org/resources/public-health-surveillance-for-cholera/>

Job Aid “Sample collection”: <https://www.gtfcc.org/resources/specimen-collection-preparation-and-packaging-for-transport/>

Job Aid “Specimen packaging and domestic transportation” :

<https://www.gtfcc.org/resources/specimen-collection-preparation-and-packaging-for-transport/>

Job Aid “Strain conditioning for international transportation”:

<https://www.gtfcc.org/resources/specimen-collection-preparation-and-packaging-for-transport/>

"Laboratory referral form" : <https://www.gtfcc.org/resources/gtfcc-laboratory-referral-and-results-reporting-forms/>



END OF MODULE ASSESSMENT



Assessment

1. It is preferable to avoid collecting a sample for cholera testing after antibiotic treatment and this may affect the quality of the sample:
True or False
2. Stool samples can be collected from bedpans that contain traces of disinfectant:
True or False
3. An unpreserved stool sample in a stool collection cup shouldn't be tested by culture in the lab after from collection:
a. 24 hours b. 7 days c. 2 hours d. 2 days
4. Cary Blair swabs should be transported at 4° C:
True or False
5. If ice packs are used during transport, they should not be placed in direct contact with the sample:
True or False

Assessment Answers

1. It is preferable to avoid collecting a sample for cholera testing after antibiotic treatment and this may affect the quality of the sample:
True but sample can still be collected, and the antibiotics recorded on the referral form to refresh your memory go to slide 7
2. Stool samples can be collected from bedpans that contain traces of disinfectant:
False disinfectant kills bacteria and will lead to bad samples to refresh your memory go to slide 11
3. An unpreserved stool sample in a stool collection cup shouldn't be tested by culture in the lab after from collection:
c. 2 hours to refresh your memory go to slide 25
4. Cary Blair swabs should be transported at 4°C:
False refrigerating samples can damage the sample give incorrect test results to refresh your memory go to slide 24
5. If ice packs are used during transport, they should not be placed in direct contact with the sample:
True to refresh your memory go to slide 37