GLOBAL TASK FORCE ON
CHOLERA CONTROL

LABORATORY WG ACHIEVEMENTS

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1. BACKGROUND

THE GLOBAL ROADMAP FROM A LABORATORY PERSPECTIVE

Three strategic focus areas have been identified in The Global Roadmap for Ending cholera

Axis 1: Early detection and quick response to contain outbreaks at an early stage

Axis 2: Multisectoral approach to prevent cholera in hotspots (PAMI) in endemic countries

Axis 3: Effective mechanism of coordination for technical support, resource mobilisation, and partnership at the local and global level.

The Laboratory Working Group develops laboratory-specific approaches and also works jointly on specific topics with the Epi group as part of the Surveillance Working Group

SPECIFIC TASKS OF THE LABORATORY WORKING GROUP

- Provide tools for technical support of laboratories (forms, job aids, fact sheets, data sheets etc…)
- Support countries to map existing capacities, and link with epidemiological data to identify laboratory needs around PAMI.
- Identify needs and in particular mechanisms for provision of equipment/supplies and establish a link with donors
- Support the training of laboratory staff in testing and testing strategies to improve performance and speed
  - At central/reference lab level
  - At peripheral level (particularly around PAMI)
- Provide elements of quality assurance and progress assessment
- Establish links with and promote regional and sub-regional laboratory networks to improve preparedness and coordination.
- Explore opportunities for potential cross-fertilisation with other disease fields and promote the strengthening of bacteriology laboratories as a whole.

LABORATORY WORKING GROUP ACTIVITIES 2023-2024

Another busy year on many different fronts, and introduction of new activities

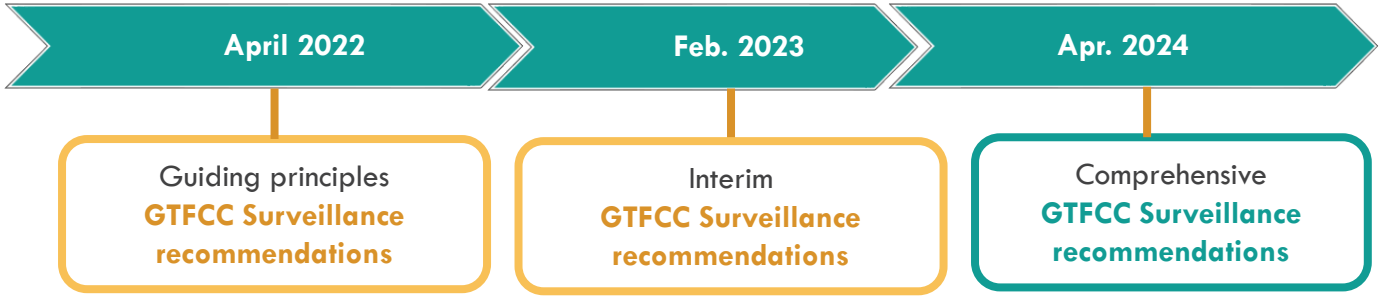
- Guidance and Tools
- New activities
  - Capacity assessment (field visits)
  - Implementation of initial training activities in the field
  - Development of support materials for training

2. ACHIEVEMENTS

LWG WORKPLAN 2023

		2023								2024					Status
Category / Thematic area	**Activities (description)**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	
Guidance development	Environmental Surveillance Technical Note														V1 published
	Minimum Standards for Lab Capacity														*activity postponed to 2025
	General Technical Guidance for PCR														*activity postponed to 2025
	Laboratory Testing Strategy														
Technical tools	AMR Fact sheet														Drafted, in design
	Stool specimen collection														Drafting
	Lab Referral Form														Drafted, in design
	Lab Reporting Form														Drafted, in design
	Form to Accompany Shipment of Isolates														Drafted, in design
Lab Capacity Assessments	Finalize Capacity Assessment Toolkit														V1 drafted
	Pilot in 4 countries														2 done, 2 in preparation
Training of Lab Personnel	Develop training plan/ materials/checklists														Drafted, in design
	Online training modules														
	Implement ToT in 4 priority countries														2 done, 2 in preparation
Surv WG Subgroup activities	Updated surveillance guidelines														Finalizing
Improving Cholera Diagnostics Initiative	RDT TPP Review														Finalized
	TPP Development Mol. Diag.														Public consultation
	Mol. Diag. Evaluation Protocol														Ongoing

PUBLIC HEALTH SURVEILLANCE FOR CHOLERA GUIDANCE DOCUMENT 2024

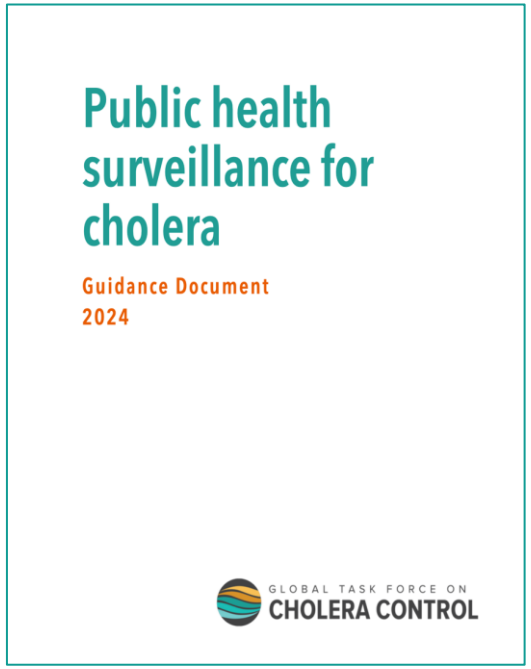


A new review has been carried out from June to September 2023 by the EPI working group

- New epi-settings considered for **adaptative** surveillance
- New definitions, added or simplified
- Revised structure of the guidance

The Laboratory WG refined specific laboratory items:

- Improving the language used for the laboratory section
- Removing the SOP aspects, intended to be included in a specific document
- Checking that the testing recommendations were still appropriate
- Adding a section on laboratory capacity



English: https://www.gtfcc.org/wp-content/uploads/2024/04/public-health-surveillance-for-cholera-guidance-document-2024.pdf
French: ongoing

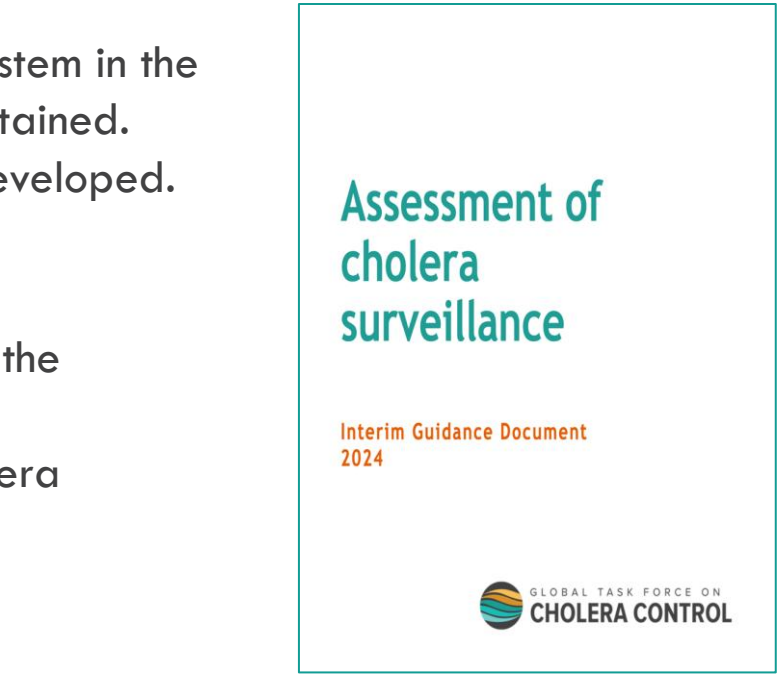
ASSESSMENT OF CHOLERA SURVEILLANCE – GUIDANCE DOCUMENT

Guidance document providing a method to assess compliance with the Surveillance Guidance

- ☞ Aims to assess the cholera surveillance strategies and the cholera surveillance system in the country and to identify critical gaps, areas of improvement, capacity to be maintained.
- ☞ To be performed as a self-assessment by countries at a minimum when NCP is developed.

From laboratory perspective

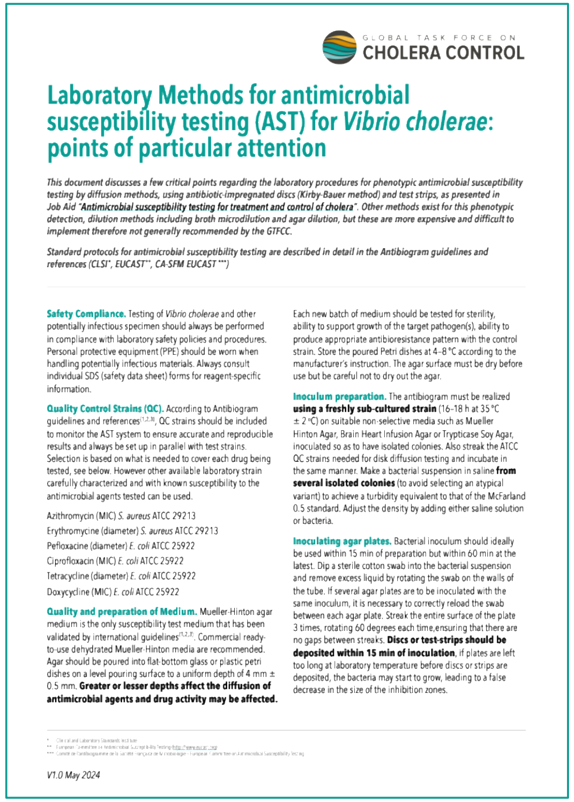
- ☞ Evaluation of laboratory capacities (reference laboratory capacity, capacity of the national laboratory system, decentralisation of laboratory surveillance)
- ☞ Evaluation of testing recommendations for early detection and monitorig of cholera outbreaks
- ☞ Based on the laboratory minimum capacities defined in this document



English: https://www.gtfcc.org/wp-content/uploads/2024/05/gtfcc-assessment-of-cholera-surveillance-en.pdf
French: ongoing

TOOLS: ANTIMICROBIAL SUSCEPTIBILITY TESTING

- Fact-sheet V1


GLOBAL TASK FORCE ON CHOLERA CONTROL

Laboratory Methods for antimicrobial susceptibility testing (AST) for *Vibrio cholerae*: points of particular attention

This document discusses a few critical points regarding the laboratory procedures for phenotypic antimicrobial susceptibility testing by diffusion methods, using antibiotic-impregnated discs (Kirby-Bauer method) and test strips, as presented in Job Aid "Antimicrobial susceptibility testing for treatment and control of cholera". Other methods exist for this phenotypic detection, dilution methods including broth microdilution and agar dilution, but these are more expensive and difficult to implement therefore not generally recommended by the GTFCC.

Standard protocols for antimicrobial susceptibility testing are described in detail in the Antibiogram guidelines and references (CLSI, EUCAST**, CA-SFM EUCAST ***)*

Safety Compliance. Testing of *Vibrio cholerae* and other potentially infectious specimen should always be performed in compliance with laboratory safety policies and procedures. Personal protective equipment (PPE) should be worn when handling potentially infectious materials. Always consult individual SDS (safety data sheet) forms for reagent-specific information.

Quality Control Strains (QC). According to Antibiogram guidelines and references[1,2,3], QC strains should be included to monitor the AST system to ensure accurate and reproducible results and always be set up in parallel with test strains. Selection is based on what is needed to cover each drug being tested, see below. However other available laboratory strain carefully characterized and with known susceptibility to the antimicrobial agents tested can be used.

Azithromycin (MIC) *S. aureus* ATCC 29213
Erythromycine (diameter) *S. aureus* ATCC 29213
Pefloxacine (diameter) *E. coli* ATCC 25922
Ciprofloxacin (MIC) *E. coli* ATCC 25922
Tetracycline (diameter) *E. coli* ATCC 25922
Doxycycline (MIC) *E. coli* ATCC 25922

Quality and preparation of Medium. Mueller-Hinton agar medium is the only susceptibility test medium that has been validated by international guidelines[1,2,3]. Commercial ready-to-use dehydrated Mueller-Hinton media are recommended. Agar should be poured into flat-bottom glass or plastic petri dishes on a level pouring surface to a uniform depth of 4 mm ± 0.5 mm. **Greater or lesser depths affect the diffusion of antimicrobial agents and drug activity may be affected.**

Each new batch of medium should be tested for sterility, ability to support growth of the target pathogen(s), ability to produce appropriate antibioresistance pattern with the control strain. Store the poured Petri dishes at 4–8 °C according to the manufacturer's instruction. The agar surface must be dry before use but be careful not to dry out the agar.

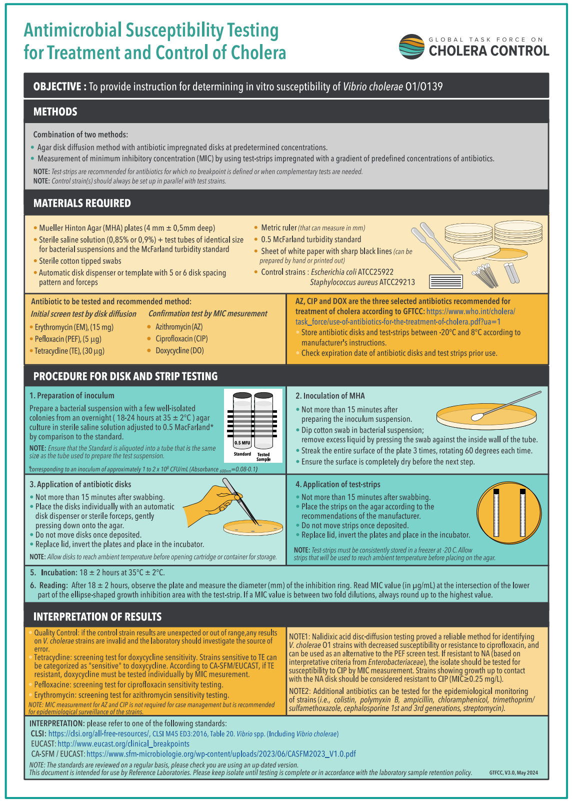
Inoculum preparation. The antibiogram must be realized **using a freshly sub-cultured strain** (16–18 h at 35 °C ± 2 °C) on suitable non-selective media such as Mueller Hinton Agar, Brain Heart Infusion Agar or Trypticase Soy Agar, inoculated so as to have isolated colonies. Also streak the ATCC QC strains needed for disk diffusion testing and incubate in the same manner. Make a bacterial suspension in saline **from several isolated colonies** (to avoid selecting an atypical variant) to achieve a turbidity equivalent to that of the McFarland 0.5 standard. Adjust the density by adding either saline solution or bacteria.

Inoculating agar plates. Bacterial inoculum should ideally be used within 15 min of preparation but within 60 min at the latest. Dip a sterile cotton swab into the bacterial suspension and remove excess liquid by rotating the swab on the walls of the tube. If several agar plates are to be inoculated with the same inoculum, it is necessary to correctly reload the swab between each agar plate. Streak the entire surface of the plate 3 times, rotating 60 degrees each time, ensuring that there are no gaps between streaks. **Discs or test-strips should be deposited within 15 min of inoculation**, if plates are left too long at laboratory temperature before discs or strips are deposited, the bacteria may start to grow, leading to a false decrease in the size of the inhibition zones.

V1.0 May 2024

- Job-aid V3

Revised in line with new recommendations

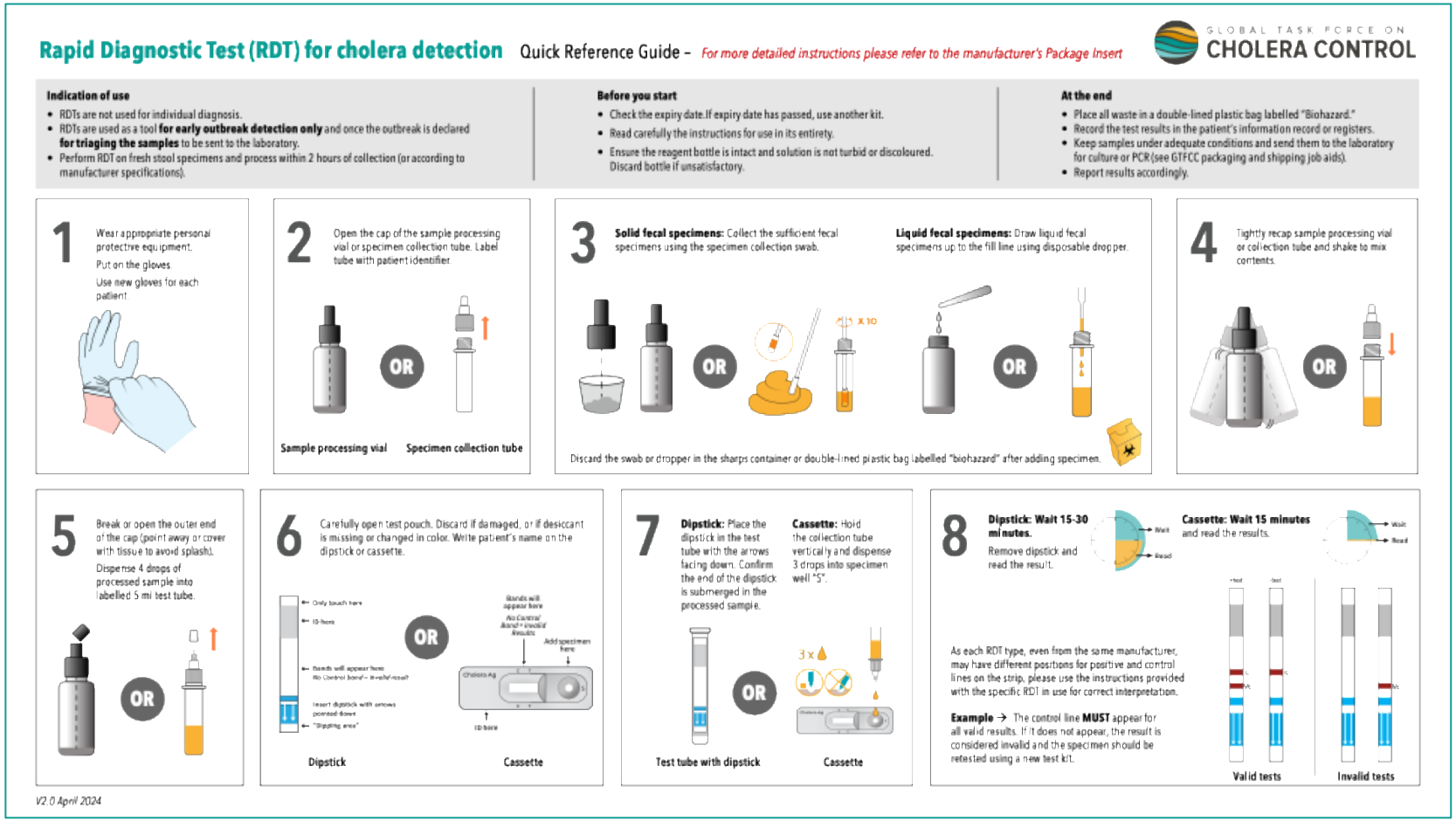


English: https://www.gtfcc.org/wp-content/uploads/2024/05/gtfcc-fact-sheet-laboratory-methods-for-ast-for-cholera-en.pdf
French: https://www.gtfcc.org/wp-content/uploads/2024/05/gtfcc-fact-sheet-laboratory-methods-for-ast-for-cholera-fr.pdf

English: https://www.gtfcc.org/wp-content/uploads/2024/03/gtfcc-job-aid-antimicrobial-susceptibility-testing-for-treatment-and-control-of-cholera-en.pdf
French: https://www.gtfcc.org/wp-content/uploads/2024/03/gtfcc-job-aid-antimicrobial-susceptibility-testing-for-treatment-and-control-of-cholera-fr.pdf

TOOLS: JOB-AID ON RDT USE

- Images updated by a designer
- Slightly revised



Rapid Diagnostic Test (RDT) for cholera detection Quick Reference Guide – *For more detailed instructions please refer to the manufacturer's Package Insert*

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Indication of use
- RDTs are not used for individual diagnosis.
- RDTs are used as a tool **for early outbreak detection only** and once the outbreak is declared **for triaging the samples** to be sent to the laboratory.
- Perform RDT on fresh stool specimens and process within 2 hours of collection (or according to manufacturer specifications).

Before you start
- Check the expiry date. If expiry date has passed, use another kit.
- Read carefully the instructions for use in its entirety.
- Ensure the reagent bottle is intact and solution is not turbid or discoloured. Discard bottle if unsatisfactory.

At the end
- Place all waste in a double-lined plastic bag labelled "Biohazard."
- Record the test results in the patient's information record or registers.
- Keep samples under adequate conditions and send them to the laboratory for culture or PCR (see GTFCC packaging and shipping job aids).
- Report results accordingly.

1 Wear appropriate personal protective equipment.
Put on the gloves.
Use new gloves for each patient.

2 Open the cap of the sample processing vial or specimen collection tube. Label tube with patient identifier.
OR
Sample processing vial — Specimen collection tube

3 **Solid fecal specimens:** Collect the sufficient fecal specimens using the specimen collection swab.
Liquid fecal specimens: Draw liquid fecal specimens up to the fill line using disposable dropper.
OR
Discard the swab or dropper in the sharps container or double-lined plastic bag labelled "biohazard" after adding specimen.

4 Tightly recap sample processing vial or collection tube and shake to mix contents.
OR

5 Break or open the outer end of the cap (point away or cover with tissue to avoid splash).
Dispense 4 drops of processed sample into labelled 5 ml test tube.
OR

6 Carefully open test pouch. Discard if damaged, or if desiccant is missing or changed in color. Write patient's name on the dipstick or cassette.
OR
Dipstick — Cassette

7 **Dipstick:** Place the dipstick in the test tube with the arrows facing down. Confirm the end of the dipstick is submerged in the processed sample.
Cassette: Hold the collection tube vertically and dispense 3 drops into specimen well "S".
OR
Test tube with dipstick — Cassette

8 **Dipstick: Wait 15-30 minutes.**
Remove dipstick and read the result.
Cassette: Wait 15 minutes and read the results.
As each RDT type, even from the same manufacturer, may have different positions for positive and control lines on the strip, please use the instructions provided with the specific RDT in use for correct interpretation.
Example → The control line **MUST** appear for all valid results. If it does not appear, the result is considered invalid and the specimen should be retested using a new test kit.
Valid tests — Invalid tests

V2.0 April 2024

English: https://www.gtfcc.org/wp-content/uploads/2022/01/gtfcc-job-aid-rapid-diagnostic-test-for-cholera-detection-en-1.pdf
French: https://www.gtfcc.org/wp-content/uploads/2023/07/gtfcc-job-aid-rapid-diagnostic-test-for-cholera-detection-fr-1.pdf

TPP FOR A RAPID DIAGNOSTIC TEST FOR SURVEILLANCE OF CHOLERA OUTBREAKS

Development of this TPP document coordinated by FIND with a financial support by Gavi
TPP development leadership team (5 personns)
TPP development focus group (13 personns)
Public consultation review and comments involving memebers of the Lab Working group
Level of agreement averaging 95%

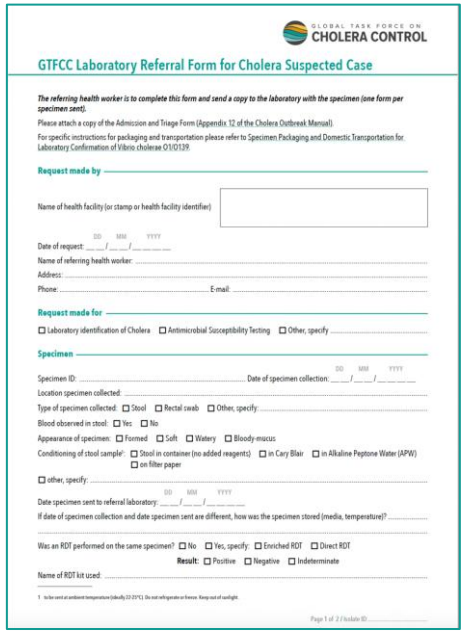
- TPP for cholera RDTs first published in 2017
- Need to update the TPP due to some criteria too stringents and advances in the field

CHARACTERISTIC	MINIMAL	PREFERRED
Clinical sensitivity[1]	≥ 90% for each assay target[2]	≥ 95% for each assay target[3]
Clinical specificity[1]	≥ 85% for each assay target[2]	≥ 95% for each assay target[3]

https://www.gtfcc.org/resources/target-product-profile-for-a-rapid-diagnostic-test-for-surveillance-of-cholera-outbreaks/

3. IN PROGRESS

DOCUMENTATION TO ACCOMPANY SHIPMENT OF ISOLATES



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 (Appendix 12 of the Cholera Outbreak Manual).

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: DD MM YYYY

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 of specimen collection: DD MM YYYY

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[1]: ☐ 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: DD MM YYYY

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

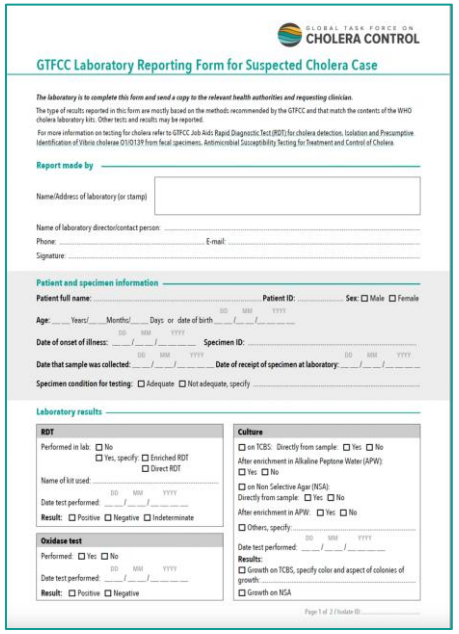
Result: ☐ Positive ☐ Negative ☐ Indeterminate

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 / Isolate ID:

- **Lab referral form:** for health care workers to fill and send to laboratories with samples collected from suspect cases. Minimal data for laboratories to perform tests and interpret test results.



GLOBAL TASK FORCE ON CHOLERA CONTROL

GTFCC Laboratory Reporting Form for Suspected Cholera Case

The laboratory is to complete this form and send a copy to the relevant health authorities and requesting clinician.

The type of results reported in this form are mostly based on the methods recommended by the GTFCC and that match the contents of the WHO cholera laboratory kits. Other tests and results may be reported.

For more information on testing for cholera refer to GTFCC Job Aids: Rapid Diagnostic Test (RDT) for cholera detection, Isolation and Presumptive Identification of Vibrio cholerae O1/O139 from fecal specimens, Antimicrobial Susceptibility Testing for Treatment and Control of Cholera.

Report made by

Name/Address of laboratory (or stamp)

Name of laboratory director/contact person:

Phone: E-mail:

Signature:

Patient and specimen information

Patient full name: Patient ID: Sex: ☐ Male ☐ Female

Age: Years/ Months/ Days or date of birth DD MM YYYY

Date of onset of illness: DD MM YYYY Specimen ID:

Date that sample was collected: DD MM YYYY Date of receipt of specimen at laboratory: DD MM YYYY

Specimen condition for testing: ☐ Adequate ☐ Not adequate, specify

Laboratory results

RDT

Performed in lab: ☐ No ☐ Yes, specify: ☐ Enriched RDT ☐ Direct RDT

Name of kit used:

Date test performed: DD MM YYYY

Result: ☐ Positive ☐ Negative ☐ Indeterminate

Oxidase test

Performed: ☐ Yes ☐ No

Date test performed: DD MM YYYY

Result: ☐ Positive ☐ Negative

Culture

☐ on TCBS: Directly from sample: ☐ Yes ☐ No

After enrichment in Alkaline Peptone Water (APW): ☐ Yes ☐ No

☐ on Non Selective Agar (NSA): Directly from sample: ☐ Yes ☐ No

After enrichment in APW: ☐ Yes ☐ No

☐ Others, specify:

Date test performed: DD MM YYYY

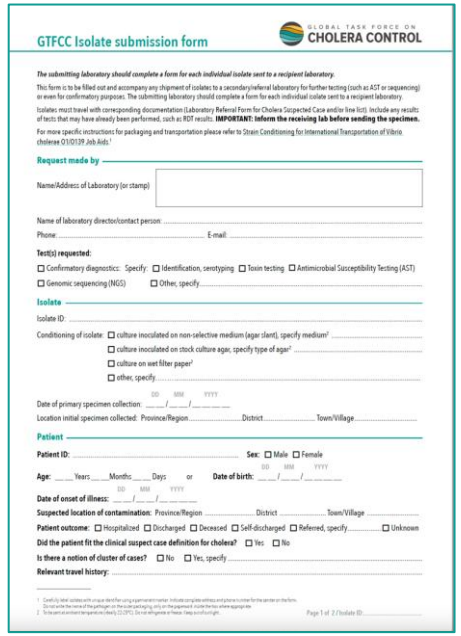
Results:

☐ Growth on TCBS, specify color and aspect of colonies of growth:

☐ Growth on NSA

Page 1 of 2 / Isolate ID:

- **Lab reporting form:** for laboratories to report results back to clinicians and/or public health authorities. To contain minimal data to be further included in National database.



GTFCC Isolate submission form

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The submitting laboratory should complete a form for each individual isolate sent to a recipient laboratory.

This form is to be filled out and accompany any shipment of isolates to a secondary/referral laboratory for further testing (such as AST or sequencing) or even for confirmatory purposes. The submitting laboratory should complete a form for each individual isolate sent to a recipient laboratory.

Isolates must travel with corresponding documentation (Laboratory Referral Form for Cholera Suspected Case and/or line list). Include any results of tests that may have already been performed, such as RDT results. **IMPORTANT: inform the receiving lab before sending the specimen.**

For more specific instructions for packaging and transportation please refer to Strain Conditioning for International Transportation of Vibrio cholerae O1/O139 Job Aids.[1]

Request made by

Name/Address of Laboratory (or stamp)

Name of laboratory director/contact person:

Phone: E-mail:

Test(s) requested:

☐ Confirmatory diagnostics: Specify: ☐ Identification, serotyping ☐ Toxin testing ☐ Antimicrobial Susceptibility Testing (AST)

☐ Genomic sequencing (NGS) ☐ Other, specify:

Isolate

Isolate ID:

Conditioning of isolate: ☐ culture inoculated on non-selective medium (agar slant), specify medium[2]

☐ culture inoculated on stock culture agar, specify type of agar[2]

☐ culture on wet filter paper[2]

☐ other, specify:

Date of primary specimen collection: DD MM YYYY

Location initial specimen collected: Province/Region District Town/Village

Patient

Patient ID: Sex: ☐ Male ☐ Female

Age: Years Months Days or Date of birth: DD MM YYYY

Date of onset of illness: DD MM YYYY

Suspected location of contamination: Province/Region District Town/Village

Patient outcome: ☐ Hospitalized ☐ Discharged ☐ Deceased ☐ Self-discharged ☐ Referred, specify ☐ Unknown

Did the patient fit the clinical suspect case definition for cholera? ☐ Yes ☐ No

Is there a notion of cluster of cases? ☐ No ☐ Yes, specify

Relevant travel history:

1 Carefully label isolates with a unique identifier using a permanent marker. Indicate complete address and phone number for the sender on the form.
Do not write the name of the pathogen on the outer packaging, only on the paperwork inside the box where appropriate.
2 To be sent at ambient temperature (ideally 22-25°C). Do not refrigerate or freeze. Keep out of sunlight.

Page 1 of 2 / Isolate ID:

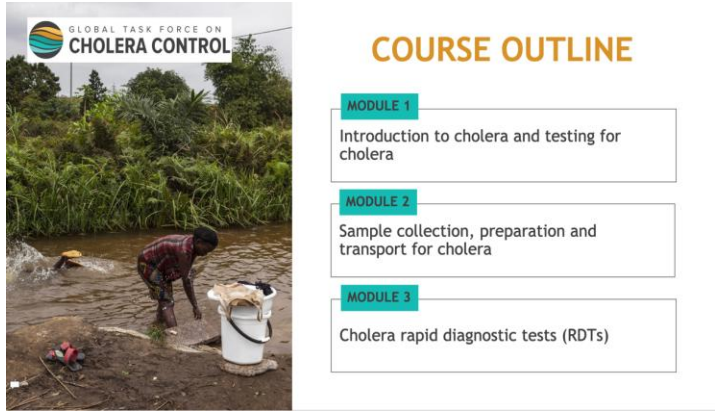
- **Isolate submission form:** for laboratories to accompany any shipment of isolates to a secondary/referral laboratory for further testing (such as AST or sequencing) or even for confirmatory purposes.

SUPPORTS FOR TRAINING : STANDARDISED TRAINING MATERIALS

Training package 1 for field staff, Module 1 “Introduction to Cholera and Testing for Cholera”

Training package 1 for field staff, Module 2 “Sample collection, Preparation and Transport for Cholera”

Training package 1 for field staff, Module 3 “Cholera Rapid Diagnostic Tests”



LABORATORY CAPACITY ASSESSMENT TOOLS

➢ A necessary tool to help identify the needs of laboratories and the actions to be developed.

- First version re-developped in 2023
- Used for field evaluation in two countries in 2023 and 2024, DRC and Cameroon
- Their use in the field has highlighted a number of difficulties
- A new version is currently developed by a new consultant.

TPP FOR MOLECULAR DIAGNOSTIC

- Document under development on the same principle as TPP for RDTs
- Conducted by FIND funded by GAVI
  - Effective surveillance requires diagnostic testing at all levels of the healthcare system, from communities and primary care to reference laboratories in public health institutions
  - Positive RDT results require confirmation by either culture or molecular methods
  - Molecular diagnostic tests can improve the quality and availability of laboratory data, easy-to-use, fit-for-purpose, well-performing, validated diagnostic tests are needed

JOB-AID STOOL SPECIMEN COLLECTION

- A focal point had been identified last year
- No draft document was provided but some comments and instructions were discussed
- This work is still ongoing

4. INTRODUCTION OF NEW ACTIVITIES

TRAINING

- Initiated in 2023 and funded by CDC and WHO IMST
- Includes the development of training materials and training of trainers in countries
- Implemented this year in an emergency context to support three countries: South Sudan, Somalia and Comoros Islands.

LAB CAPACITY ASSESSMENT

- Re-initiated in 2023.
- Funded by CDC
- Included the development of capacity assessment tools and the piloting of assessment in 4 priority countries
- First set of tools developped
- Two countries were visited

5. NEXT STEPS

- Continuation of ongoing activities, finalization of documents
- Large diffusion of documents and guidelines produced
- Definition of a new working plan for the coming year based on discussions and exchanges at this meeting
- Call for more Focus people to draft documents

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THANK YOU