GLOBAL TASK FORCE ON
CHOLERA CONTROL

LABORATORY WORKING GROUP DAY 3

GTFCC Epi/Lab Working Group
15–17 April 2019

OBJECTIVES OF THE SESSIONS

Review, finalize, validate Lab JOB AIDS (5) identified as priorities during the last meeting in April 2018

Discuss TECHNICAL GUIDANCE

- EQA of national labs
- PCR: appropriate techniques for molecular identification
- RDT cutoffs Pre-Qualification – Assessment of submission

Discuss a GLOBAL DATABASE

JOB AIDS

AMR testing : Validated ✓

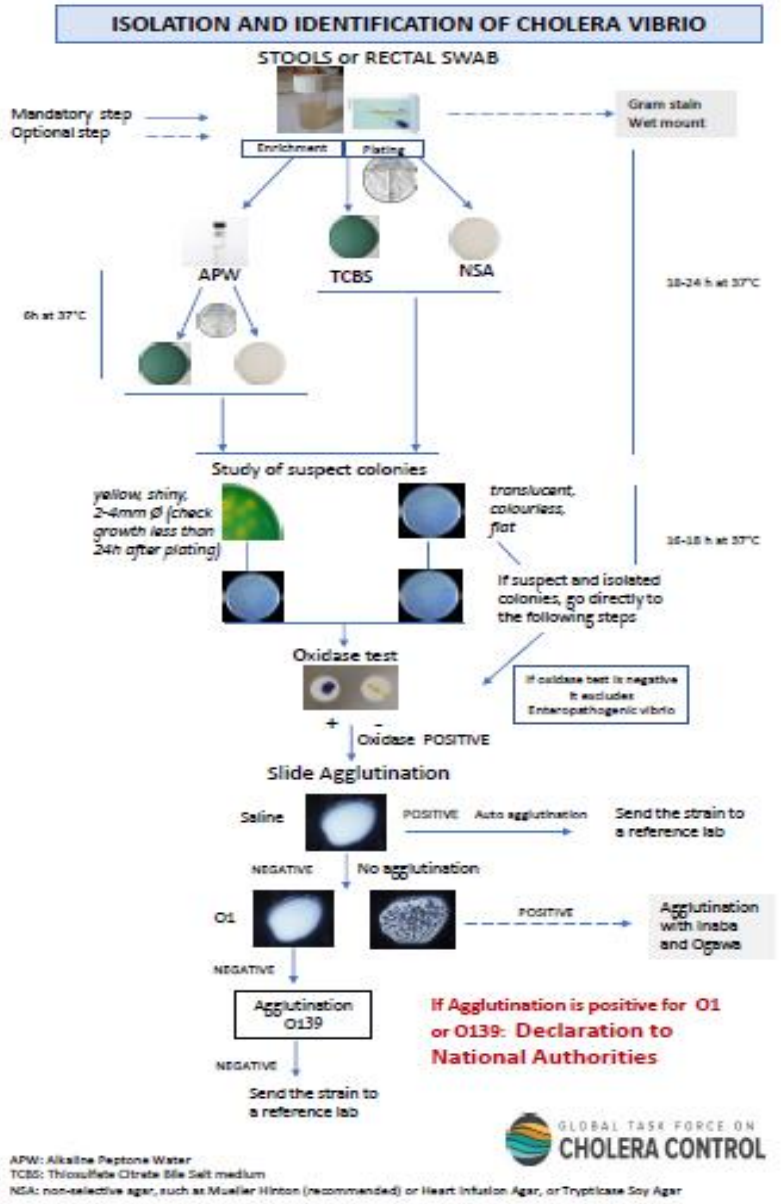
The discussion was on the antibiotics to be tested and interpretative criteria

- Test the antibiotics recommended by GTFCC for treatment as a priority (3 antibiotics + Nalidixic Acid?)
- Add a supplementary table with antibiotics to be tested for strain monitoring and surveillance
- Necessity to gather values on Azithromycine and submit them to EUCAST and CLSI for review and official recommendations
- Add a section on trouble shooting tips

JOB AIDS

Culture: Validated ✓

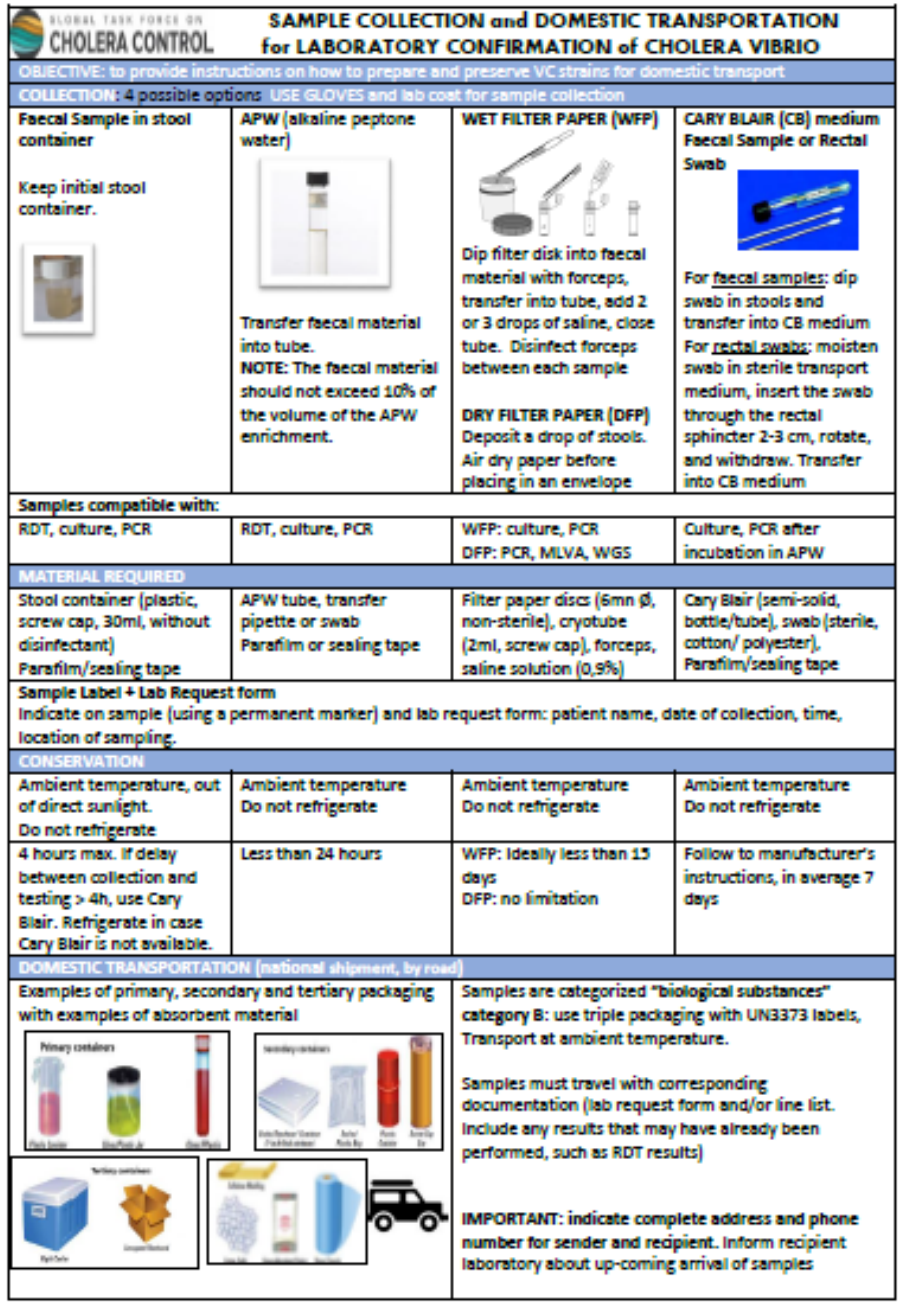
With minor edits



JOB AIDS

Domestic transportation: Validated

With minor edits



GLOBAL TASK FORCE ON CHOLERA CONTROL

SAMPLE COLLECTION and DOMESTIC TRANSPORTATION for LABORATORY CONFIRMATION of CHOLERA VIBRIO

OBJECTIVE: to provide instructions on how to prepare and preserve VC strains for domestic transport

COLLECTION: 4 possible options USE GLOVES and lab coat for sample collection

Faecal Sample in stool container Keep initial stool container.	**APW (alkaline peptone water)**	**WET FILTER PAPER (WFP)**	**CARY BLAIR (CB) medium Faecal Sample or Rectal Swab**
	Transfer faecal material into tube. **NOTE:** The faecal material should not exceed 10% of the volume of the APW enrichment.	Dip filter disk into faecal material with forceps, transfer into tube, add 2 or 3 drops of saline, close tube. Disinfect forceps between each sample **DRY FILTER PAPER (DFP)** Deposit a drop of stools. Air dry paper before placing in an envelope	For faecal samples: dip swab in stools and transfer into CB medium For rectal swabs: moisten swab in sterile transport medium, insert the swab through the rectal sphincter 2-3 cm, rotate, and withdraw. Transfer into CB medium
Samples compatible with:			
RDT, culture, PCR	RDT, culture, PCR	WFP: culture, PCR DFP: PCR, MLVA, WGS	Culture, PCR after incubation in APW
MATERIAL REQUIRED			
Stool container (plastic, screw cap, 30ml, without disinfectant) Parafilm/sealing tape	APW tube, transfer pipette or swab Parafilm or sealing tape	Filter paper discs (6mn Ø, non-sterile), cryotube (2ml, screw cap), forceps, saline solution (0,9%)	Cary Blair (semi-solid, bottle/tube), swab (sterile, cotton/ polyester), Parafilm/sealing tape
Sample Label + Lab Request form Indicate on sample (using a permanent marker) and lab request form: patient name, date of collection, time, location of sampling.			
CONSERVATION			
Ambient temperature, out of direct sunlight. Do not refrigerate	Ambient temperature Do not refrigerate	Ambient temperature Do not refrigerate	Ambient temperature Do not refrigerate
4 hours max. If delay between collection and testing > 4h, use Cary Blair. Refrigerate in case Cary Blair is not available.	Less than 24 hours	WFP: Ideally less than 15 days DFP: no limitation	Follow to manufacturer's instructions, in average 7 days
DOMESTIC TRANSPORTATION (national shipment, by road)			
Examples of primary, secondary and tertiary packaging with examples of absorbent material		Samples are categorized "biological substances" category B: use triple packaging with UN3373 labels, Transport at ambient temperature. Samples must travel with corresponding documentation (lab request form and/or line list. Include any results that may have already been performed, such as RDT results) **IMPORTANT:** indicate complete address and phone number for sender and recipient. Inform recipient laboratory about up-coming arrival of samples	

JOB AIDS

Strain conditionning for international transportation: Validate

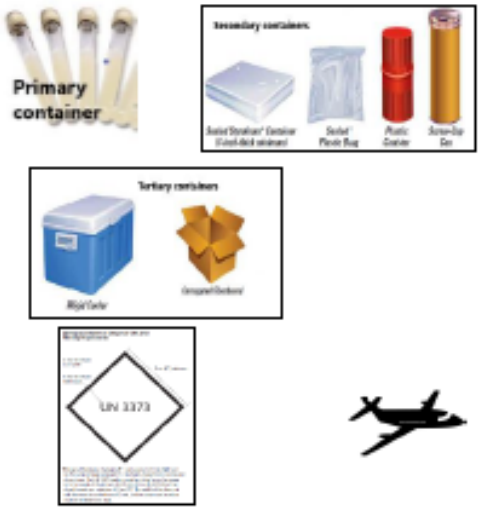
With minor edits

STRAIN CONDITIONING FOR INTERNATIONAL TRANSPORTATION of VIBRIO CHOLERAE O1 or O139

GLOBAL TASK FORCE ON CHOLERA CONTROL

OBJECTIVE: to provide instructions on how to prepare and preserve VC strains for domestic transport

STRAIN CONDITIONING (3 proposed options)[1]

Culture on WET FILTER PAPER (WFP)	Culture inoculated on NON-SELECTIVE MEDIUM	Culture inoculated on STOCK CULTURE AGAR
Material required		
Dip filter paper disk into faecal material with forceps[2], transfer into tube, add 2 or 3 drops of saline, close tube. 	Incubate slant agar in tube Tightly cap after inoculation 	Semi solid medium in tube Tightly cap after inoculation Transfer a heavily loaded loop to the tube (using an inoculating needle), then incubate to obtain growth.
CONSERVATION		
Ambient temperature. Seal with tape or parafilm. Do not refrigerate	Ambient temperature. Seal with tape or parafilm. Keep away from sunlight. Do not refrigerate	Ambient temperature. Seal with tape or parafilm. Keep away from sunlight. Do not refrigerate
No more than 2 weeks	Months	Years
Strain Label and Lab Request form Indicate: patient name or ID number, date of collection/growth, location of sampling		
INTERNATIONAL TRANSPORTATION (by air)		
Examples of primary, secondary and tertiary containers with examples of absorbent material 		The shipment by air must comply with local, national, and international regulations. Import permits, export licenses and local or national authorization may be required. For all media, follow IATA[3] regulations for **biological substances category B** and use UN3373 labels with triple packaging **IMPORTANT: indicate complete address and phone number for sender and recipient** All strains must travel with corresponding documentation (lab request form): indicate requested type of testing The accompanying forms should be placed between the secondary and tertiary container. Inform recipient laboratory about up-coming arrival of samples and provide any relevant shipping tracking details.

[1] Strains can also be frozen at -80° in liquid nitrogen but this method is not recommended for transportation because of its sophistication and its costs
[2] Disinfect forceps between each sample
[3] International Air Transport Association

JOB AIDS

RAPID DIAGNOSTIC TEST (RDT) FOR CHOLERA DETECTIO

The discussion was on generic vs individual branded product user aids

RAPID DIAGNOSTIC TEST (RDT) FOR CHOLERA DETECTION

Quick Reference Guide

Disclaimer: This is a generic reference guide. For specific instructions please always refer to the manufacturer's package insert

GLOBAL TASK FORCE ON CHOLERA CONTROL

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 screening samples** to be sent to the laboratory.
- Perform RDT on fresh stool specimens and process within 2 hours of collection.

Before you start

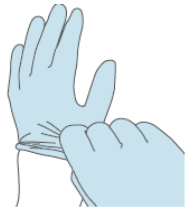
- Check the expiry date. If passed, use another kit.
- Carefully read the **manufacturer's instructions** for use in its entirety.
- Ensure the reagent bottle is intact and solution is not turbid or discoloured. Discard bottle if unsatisfactory.

At the end

- Place all waste in a double-lined plastic bag labelled "Biohazard."
- Record the test results in the patient's registers and report results accordingly.
- Send the RDT-positive samples to the reference laboratory for confirmation by culture or PCR.

1

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



2

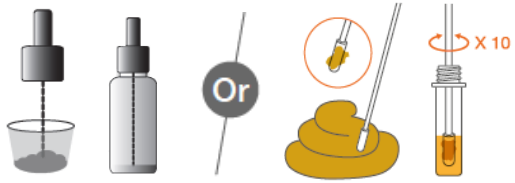
Label the sample processing vial or specimen collection tube with the patient name or ID.
Open the cap t en.

Sample processing vial

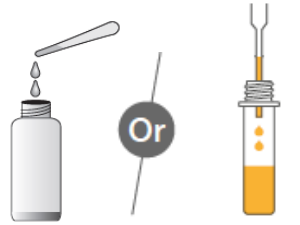
Specimen collection tube

3

Solid, semisolid or viscous stool: Use the sampling swab to collect a small portion of stool from two or more areas in the sample and insert in the sample processing vial or collection tube



Liquid stool: use disposable pipette to add liquid fecal specimen into the processing vial or specimen collection tube



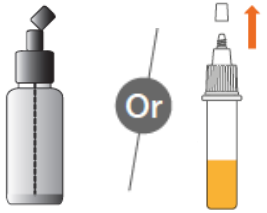
Discard the swab or dropper in the sharps container or double-lined plastic bag labelled "biohazard" after adding specimen

4

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

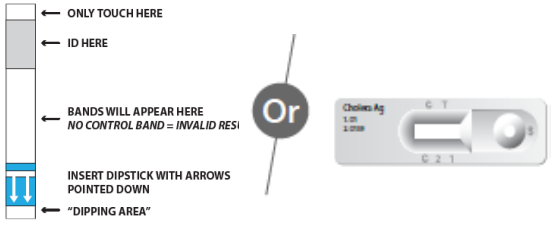
5

Break or open the outer end of the cap. Dispense 4 drops of processed sample into labelled 5 ml test tube



6

Carefully open test pouch. Discard if damaged, or if desiccant is missing or changed in color. Write the ID or patient's name on the dipstick or test device



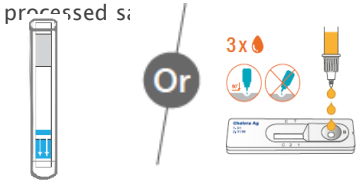
Dipstic k

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 sa

Cassette: Hold the collection tube vertically and dispense 3 drops into specimen well "S"



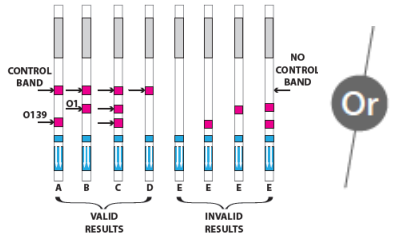
Test tube with dipstick

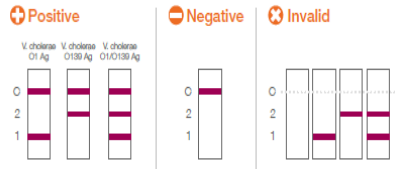
Cassette

8

Dipstick: Wait 15-minutes.
Remove dipstick and read the result

Cassette: Interpret test results **within 15 minutes** after adding Specimens and read the results





The control line should appear for all results. If it does not appear, the result is considered invalid and the specimen should be retested using a new test kit

PCR

Validated ✓ *with some edits*

- Specifically for the confirmation of diagnostic in the national lab
- Based on conventional methods, as the other methods are not f
- Encourage the use of the cheapest method for DNA extraction (
- Recommended targets:
  - Species identification
  - Cholera toxin gene
- Link to Validated protocols with respective SOPs (GTFCC Website, Trai

EQA FOR *V. CHOLERAE* IN NATIONAL LABORATORIES

- Draft technical document
  - Objectives, organization and composition EQA panel
- Use of existing EQA programmes (e.g. WHO/AFRO – NICD)
  - Restrictions in shipment of *Vibrio cholerae* as part of EQA panel
- Alternatively, EQA « inverse »
- Not only for National laboratories but open to all laboratories

RDT PRE-QUALIFICATION

Questions were on the Specificity and Sensitivity values to adopt for Prequalication

- According to GTFCC TPP? (acceptable sens $\geq$90% / spe $\geq$95%)

or

- According to GTFCC interim note? (sens $\geq$90% /spe $\geq$85%)

☛ Agreement to adopt the acceptable values recommended by the GTFCC group (interim guidance note),

- Sensitivity $\geq$ 90%
- Specificity $\geq$ 85%

GLOBAL DATABASE

- All published sequences are open access, but question of « real time surveillance »
- Which data should be included ?
  - Metadata : Specimen: place and date of isolation
  - Are genomic data sufficient for surveillance? What about laboratory data such as AMR patterns for exemple?
- Embrace community around data and discussion
  - Discussion forum website
- Tools:
  - EDGE: www.edgebioinformatics.org
  - Microreact: https://microreact.org/project/globalcholera