GLOBAL TASK FORCE ON
CHOLERA CONTROL

LABORATORY WORKING GROUP DAY 2

GTFCC Epi/Lab Working Group
15–17 April 2019

Lab WG participants

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OBJECTIVES OF THE SESSION (MORNING)

Review, finalize, validate Lab JOB AIDS which were identified as priorities during the last meeting in April 2018 and developed following a suggestion by the CDC

- RDT use and interpretation
- Sample Collection and Transportation within country
- Culture Isolation-identification of cholera vibrio
- AMR testing
- Strain Conditioning for International Transportation

OBJECTIVES OF THE SESSION (AFTERNOON)

Discuss TECHNICAL GUIDANCE

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

RAPID DIAGNOSTIC TEST (RDT) FOR CHOLERA DETECTION

Quick Reference Guide

GLOBAL TASK FORCE ON CHOLERA CONTROL

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

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

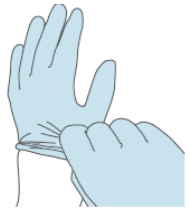
- Check the expiry date. If passed, use another kit.
- Carefully read the **manufacturer's instructions** for use in its entirety.
- Ensure the reagent bottle is intact and solution is not turbid or discoloured. Discard bottle if unsatisfactory.

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

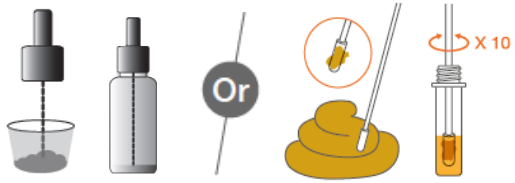
Label the sample processing vial or specimen collection tube with the patient name or ID.
Open the cap t en.

Sample processing vial

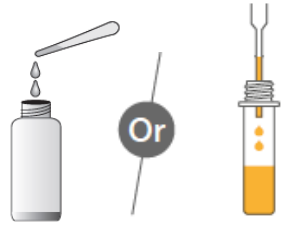
Specimen collection tube

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



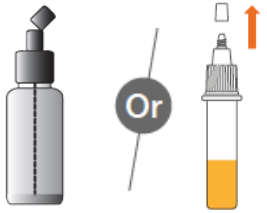
Discard the swab or dropper in the sharps container or double-lined plastic bag labelled "biohazard" after adding specimen

4

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

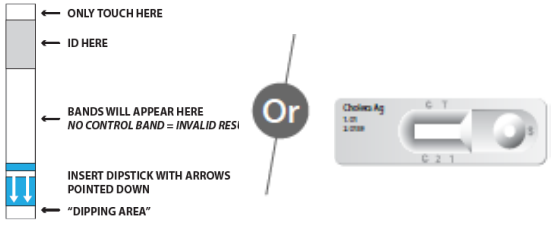
5

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



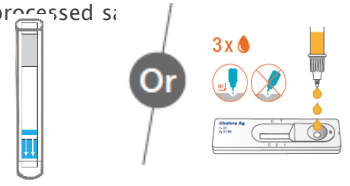
Dipstic k

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"



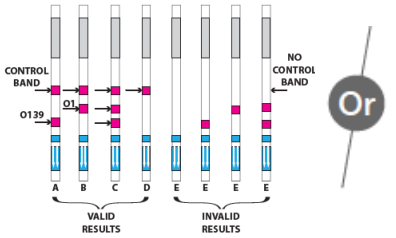
Test tube with dipstick

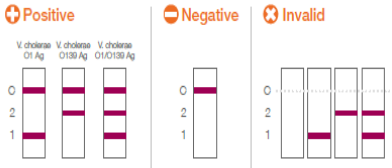
Cassette

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





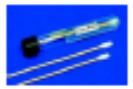
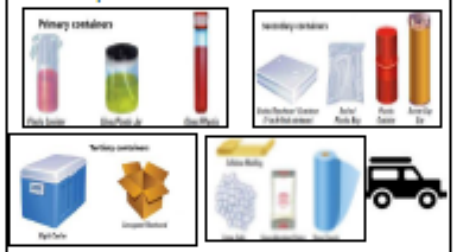
The control line should appear for all results. If it does not appear, the result is considered invalid and the specimen should be retested using a new test kit

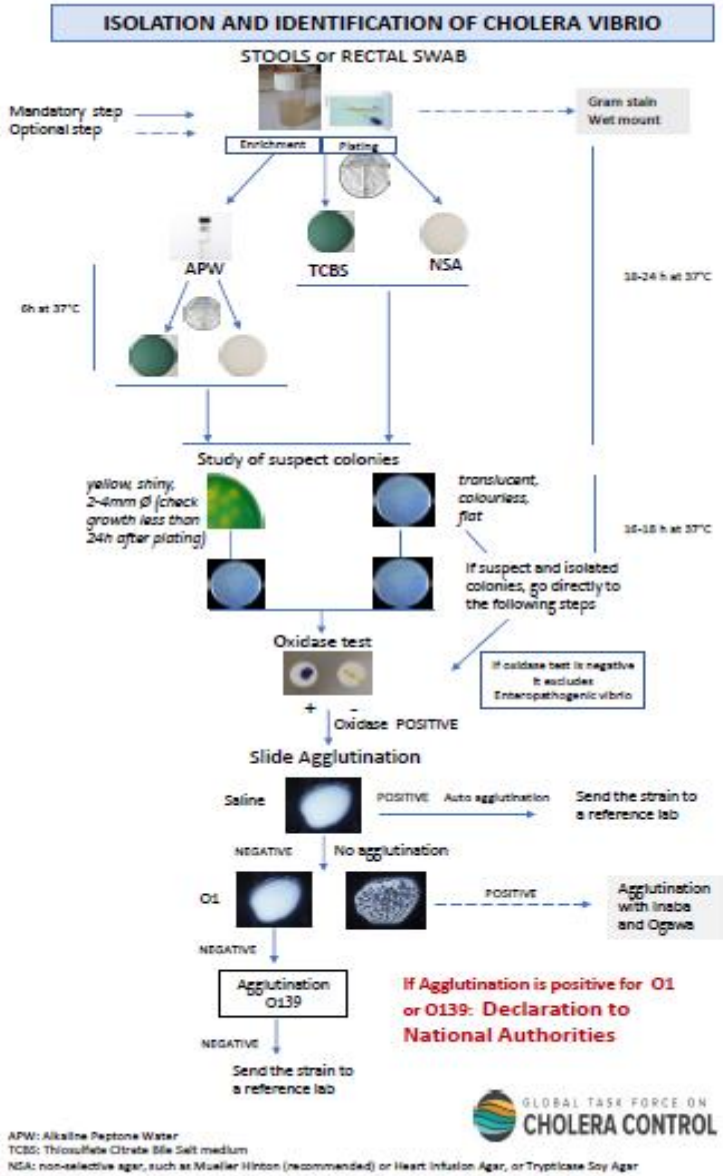
GLOBAL TASK FORCE ON CHOLERA CONTROL

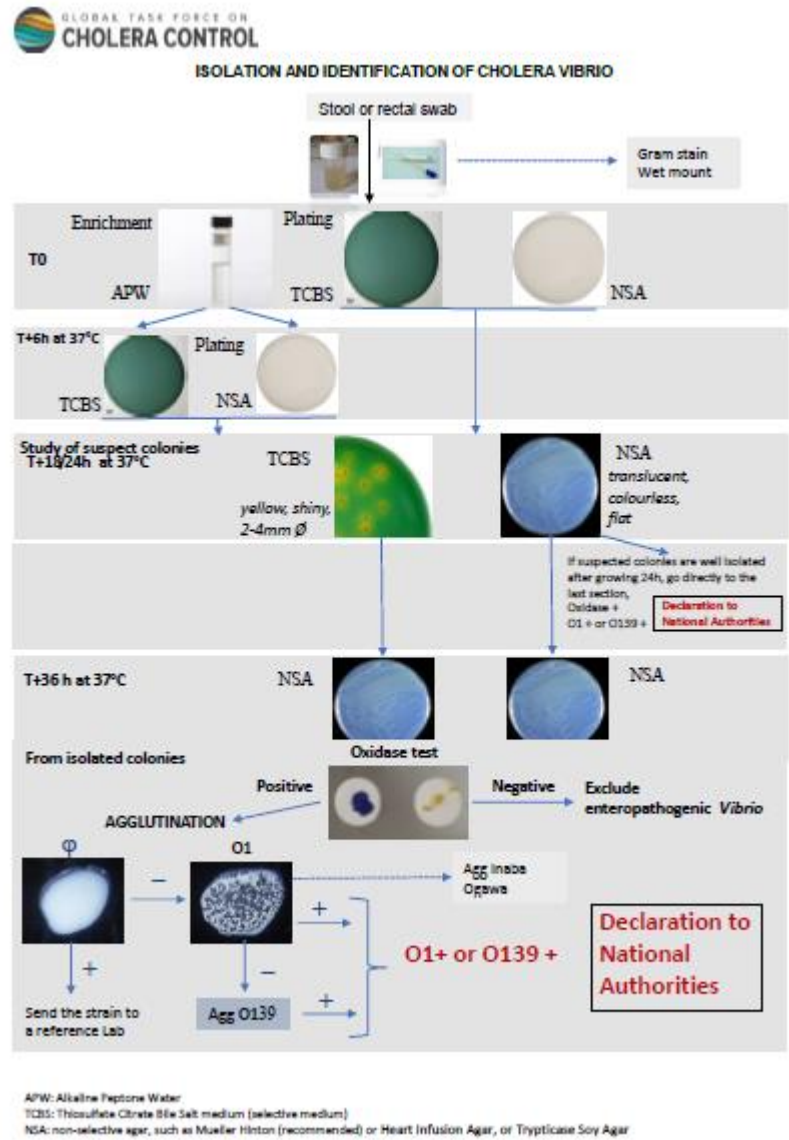
SAMPLE COLLECTION and DOMESTIC TRANSPORTATION for LABORATORY CONFIRMATION of CHOLERA VIBRIO

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)**  Transfer faecal material into tube. **NOTE:** The faecal material should not exceed 10% of the volume of the APW enrichment.	**WET FILTER PAPER (WFP)**  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	**CARY BLAIR (CB) medium** **Faecal Sample or Rectal Swab**  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**	


ISOLATION AND IDENTIFICATION OF CHOLERA VIBRIO
STOOLS or RECTAL SWAB
Mandatory step
Optional step
Enrichment
Plating
Gram stain
Wet mount
APW
TCBS
NSA
6h at 37°C
18-24 h at 37°C
Study of suspect colonies
yellow, shiny, 2-4mm Ø (check growth less than 24h after plating)
translucent, colourless, flat
16-18 h at 37°C
If suspect and isolated colonies, go directly to the following steps
Oxidase test
If oxidase test is negative it excludes Enteropathogenic vibrio
+
-
Oxidase POSITIVE
Slide Agglutination
Saline
POSITIVE
Auto agglutination
Send the strain to a reference lab
NEGATIVE
No agglutination
O1
POSITIVE
Agglutination with Inaba and Ogawa
NEGATIVE
Agglutination O139
NEGATIVE
Send the strain to a reference lab
If Agglutination is positive for O1 or O139: Declaration to National Authorities
GLOBAL TASK FORCE ON CHOLERA CONTROL
APW: Alkaline Peptone Water
TCBS: Thiosulfate Citrate Bile Salt medium
NSA: non-selective agar, such as Mueller Hinton (recommended) or Heart Infusion Agar, or Trypticase Soy Agar


GLOBAL TASK FORCE ON CHOLERA CONTROL
ISOLATION AND IDENTIFICATION OF CHOLERA VIBRIO
Stool or rectal swab
Gram stain
Wet mount
Enrichment
T0
APW
Plating
TCBS
NSA
T+6h at 37°C
TCBS
Plating
NSA
Study of suspect colonies
T+18/24h at 37°C
TCBS
yellow, shiny, 2-4mm Ø
NSA
translucent, colourless, flat
If suspected colonies are well isolated after growing 24h, go directly to the last section, Oxidase + O1 + or O139 +
Declaration to National Authorities
T+36 h at 37°C
NSA
NSA
Oxidase test
From isolated colonies
Positive
AGGLUTINATION
Negative
Exclude enteropathogenic Vibrio
O1
Agg Inaba Ogawa
Send the strain to a reference Lab
Agg O139
O1+ or O139 +
Declaration to National Authorities
APW: Alkaline Peptone Water
TCBS: Thiosulfate Citrate Bile Salt medium (selective medium)
NSA: non-selective agar, such as Mueller Hinton (recommended) or Heart Infusion Agar, or Trypticase Soy Agar

GLOBAL TASK FORCE ON CHOLERA CONTROL

ANTIMICROBIAL RESISTANCE TESTING for TREATEMENT AND CONTROL OF CHOLERA

OBJECTIVE: to provide instruction for determining in vitro susceptibility of Vibrio cholera O1/O139

METHODS

Combination of two methods:

- Disk diffusion method with antibiotic impregnated discs
- E-tests for measurement of minimum inhibitory concentration (MIC). E-tests are recommended for antibiotics for which no breakpoint is defined or complementary tests are needed)

Note: Control strain(s) should always be set up in parallel with test strains

MATERIAL REQUIRED

- Mueller Hinton Agar (MHA) plates (4 mm ± 0,5mm deep)
- Sterile saline solution (0,9%) + test tubes of comparable size to McFarland standard
- Sterile cotton tipped swabs
- Automatic disk dispenser or template with 5 or 6 disk spacing pattern and forceps
- Incubator (35°C ± 2°C)
- Metric ruler (that can measure in mm)
- 0.5 McFarland turbidity standard
- Control strain : *Escherichia coli* ATCC 25922

Store between 8°C and -20°C, (-20°C preferably for ampicillin)

Antibiotic disks	Potency
Ampicillin	10 µg
Chloramphenicol	30 µg
Nalidixic acid	30 µg
Trimethoprim/Sulfamethoxazole	1,2 /23,75 µg
Tetracycline	30 µg

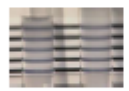
E-tests
Azithromycin
Ciprofloxacin*

* used to monitor diminished fluoroquinolone susceptibility for strains resistant to nalidixic acid

PROCEDURE for DISK and ETEST

1. Preparation of inoculum

Prepare a bacterial suspension from an overnight (18-24 hour) agar culture in sterile saline solution adjusted to 0,5 MacFarland



2. Inoculation of MHA

Dip cotton swab in bacterial suspension; remove excess liquid. Streak the entire surface of the plate 3 times, rotating 60 degrees each time. Ensure the surface is completely dry before the next step.

3. Application of antibiotic disks

Not more than 15 minutes after swabbing.
Do not move disks once deposited.
NOTE: Allow disks to reach ambient temperature before opening cartridge or container for storage.
Replace lid, invert the plates and place in the incubator.

4. Alternatively Application of E strip

Refer to the recommendations of the manufacturer
Replace lid, invert the plates and place in the incubator.

5. **Incubation**: 18h at 37°C.
6. **Reading**: After 18 hrs, observe the plate and measure the diameter (mm) of the inhibition ring.

INTERPRETATION of RESULTS (using EUCAST Enterobacteriaceae, 2018 http://www.eucast.org/clinical_breakpoints/ and CLSI guidelines, 2015 https://clsi.org/meetings/past/)

ANTIBIOTIC	Zone diameter (mm)		ETEST	CMI (mg/L)	
	S ≥	R <		S ≤	R>
Ampicillin	14	14	Azithromycin	16	
Chloramphenicol	17	17	Ciprofloxacin	0,25	0,5
Nalidixic acid	14	14			
Trimethoprim/Sulfamethoxazole	14	11			
Tetracycline (to be discussed)					
Ciprofloxacin	26	24			

Additional antibiotics can be tested for surveillance purposes (i.e. colistin, polymyxin B) or for the epidemiological monitoring of strains; according to procedures in force at the national level when existing.

GLOBAL TASK FORCE ON CHOLERA CONTROL

ANTIMICROBIAL RESISTANCE TESTING for TREATEMENT AND CONTROL OF CHOLERA

Objective: to provide instruction for determining in vitro susceptibility of Vibrio cholera O1/O139

METHODS

Combination of two methods:

- Disk diffusion method with antibiotic impregnated discs
- E-tests for measurement of minimum inhibitory concentration (MIC). NOTE: E-tests are recommended for antibiotics for which no breakpoint is defined or complementary tests are needed)

Note: Control strain(s) should always be set up in parallel with test strains

MATERIAL REQUIRED

- Mueller Hinton Agar (MHA) plates (4 mm ± 0,5mm deep)
- Sterile saline solution (0,85%) + test tubes of comparable size to McFarland standard
- Sterile cotton tipped swabs
- Automatic disk dispenser or template with 5 or 6 disk spacing pattern and forceps
- Incubator (35°C ± 2°C)
- Metric ruler (that can measure in mm)
- 0.5 McFarland turbidity standard
- Control strain : *Escherichia coli* ATCC 25922

Antibiotic disks	Potency
Ciprofloxacin	5 µg
Tetracycline	30 µg

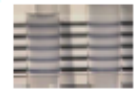
E-tests
Azithromycin

These three antibiotics are the ones recommended for treatment of cholera according to GFTCC: https://www.who.int/cholera/task_force/use-of-antibiotics-for-the-treatment-of-cholera.pdf?ua=1
Store antibiotic disks and Etests between 8°C and -20°C

Procedure for DISK and ETEST

1. Preparation of inoculum

Prepare a bacterial suspension from an overnight (18-24 hour) agar culture in sterile saline solution adjusted to 0,5 MacFarland



2. Inoculation of MHA

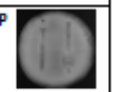
Dip cotton swab in bacterial suspension; remove excess liquid. Streak the entire surface of the plate 3 times, rotating 60 degrees each time. Ensure the surface is completely dry before the next step.

3. Application of antibiotic disks

Not more than 15 minutes after swabbing.
Do not move disks once deposited.
NOTE: Allow disks to reach ambient temperature before opening cartridge or container for storage.
Replace lid, invert the plates and place in the incubator.

4. Alternatively Application of E strip

Refer to the recommendations of the manufacturer
Replace lid, invert the plates and place in the incubator.



5. **Incubation**: 18h at 37°C.
6. **Reading**: After 18 hrs, observe the plate and measure the diameter (mm) of the inhibition ring.

INTERPRETATION of RESULTS (using CLSI guidelines, M45 Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria, 2015)

ANTIBIOTIC	Zone diameter (mm)			ETEST	CMI (mg/L)
	S ≥	I	R ≤	S ≤	R>
Ciprofloxacin	≥ 21	16-20	≤ 15		
Tetracycline	≥ 15	12-14	≤ 11		
Azithromycin				≤ 2	-

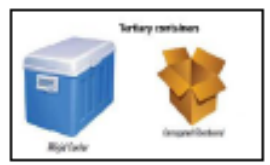
Additional antibiotics can be tested for surveillance purposes or for the epidemiological monitoring of strains; according to procedures in force at the national level when existing.

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

EQA FOR CHOLERA IN NATIONAL LABORATORIES

Goal

- Improve the quality of laboratory diagnostics of the national laboratories in cholera affected countries
  - Early detection and confirmation of outbreaks
  - Monitoring the circulation of cholera vibrios

Objectives

- Assess the quality of laboratory performance for identification and characterization of cholera vibrio strains
- Identify common errors and recommend corrective measures
- Encourage good laboratory practice, the implementation of quality assurance and stimulate information exchange and networking among laboratories

EQA FOR CHOLERA OF NATIONAL LABORATORIES

- Survey conducted in 2018 in eight cholera-endemic countries to assess their surveillance capacities, including participation in EQA programmes
  - None of the eight countries reported having collaborations or agreements with international laboratories for conducting EQA for cholera
  - Requested from national labs to improve their quality – GTFCC « Laboratory Package » (Offer of Service)
- WHO/AFRO Regional Laboratory External Quality Assessement Programme (EQAP)
  - Established in 2002 to monitor the performance of public health laboratories when diagnosing epidemic-prone baterial diseases - Identification, culture, serotyping and AST
  - Provided by the National Health Laboratory Service at NICD in South Africa with support of « referee laboratories »
  - The only EQAP that covers general bacteriology in Africa that is freely available to national labs
  - As of 2016, 82 laboratories from 46 countries participated
  - However, limitation in sending cultures of *Vibrio cholera* as part of the EQA panel due to adherence to South African legislation

EQA FOR CHOLERA IN NATIONAL LABORATORIES

- How can we use the existing EQAP provided by NICD for cholera (considering the current limitation for shipment of cholera vibrio strains)?
- Alternative EQA “inverse”
- Role of the GTFCC and International labs (IP, CDC, etc.)
- EQA Panel
  - EQA samples: positive and negative *Vibrio cholerae* isolates
  - number of samples per panel and frequency of surveys
- EQA of *Vibrio cholerae* identification (culture or PCR)
  - Confirmation of the *Vibrio cholerae* species
  - Determination of Serogroups: O1 or O139,
  - Optional: determination of serotypes (Inaba, Ogawa) and AST

PCR

- Objective
- Target audience
- Use of PCR tests for surveillance purposes
- Principles of PCR tests
- Target sequences used in PCR assays
- Methods for DNA extraction prior PCR testing
- How to select the best adapted assays according to the country’s needs

RDT PRE-QUALIFICATION

Presentation overview (Anne-Laure PAGE)

- PQ scope and components
- PQ process
- PQ decision
- Post-PQ activities
- PQ of assays for the detection of *V. cholerae*
- Pass/fail criteria for PQ of cholera assays (performance evaluation)

GLOBAL DATABASE

Open discussions