



**Protocol for the performance evaluation of  
rapid diagnostic tests for the detection of  
*Vibrio cholerae* O1 or O1/O139  
for WHO prequalification assessment**

**Prequalification Unit  
In Vitro Diagnostics Assessment Team**

Protocol for the performance evaluation of rapid diagnostic tests for the detection of *Vibrio cholerae* O1 or O1/O139 for WHO prequalification assessment, PQDx\_305 version 2

ISBN 978-92-4-007504-7 (electronic version)

ISBN 978-92-4-007505-4 (print version)

© World Health Organization 2023

Some rights reserved. This work is available under the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 IGO licence (CC BY-NC-SA 3.0 IGO; <https://creativecommons.org/licenses/by-nc-sa/3.0/igo>).

Under the terms of this licence, you may copy, redistribute and adapt the work for non-commercial purposes, provided the work is appropriately cited, as indicated below. In any use of this work, there should be no suggestion that WHO endorses any specific organization, products or services. The use of the WHO logo is not permitted. If you adapt the work, then you must license your work under the same or equivalent Creative Commons licence. If you create a translation of this work, you should add the following disclaimer along with the suggested citation: “This translation was not created by the World Health Organization (WHO). WHO is not responsible for the content or accuracy of this translation. The original English edition shall be the binding and authentic edition”. Any mediation relating to disputes arising under the licence shall be conducted in accordance with the mediation rules of the World Intellectual Property Organization (<http://www.wipo.int/amc/en/mediation/rules/>).

**Suggested citation.** Protocol for the performance evaluation of rapid diagnostic tests for the detection of *Vibrio cholerae* O1 or O1/O139 for WHO prequalification assessment, PQDx\_305 version 2. Geneva: World Health Organization; 2023. Licence: [CC BY-NC-SA 3.0 IGO](https://creativecommons.org/licenses/by-nc-sa/3.0/igo).

**Cataloguing-in-Publication (CIP) data.** CIP data are available at <http://apps.who.int/iris>.

**Sales, rights and licensing.** To purchase WHO publications, see <https://www.who.int/publications/book-orders>. To submit requests for commercial use and queries on rights and licensing, see <https://www.who.int/copyright>.

**Third-party materials.** If you wish to reuse material from this work that is attributed to a third party, such as tables, figures or images, it is your responsibility to determine whether permission is needed for that reuse and to obtain permission from the copyright holder. The risk of claims resulting from infringement of any third-party-owned component in the work rests solely with the user.

**General disclaimers.** The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of WHO concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by WHO in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by WHO to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall WHO be liable for damages arising from its use.

## Contents

1.	Introduction .....	5
1.1	WHO Prequalification of In Vitro Diagnostics .....	5
1.2	WHO performance evaluation of rapid diagnostic tests for the detection of <i>Vibrio cholerae</i> O1 or O1/O139 .....	5
2.	Intended audience .....	5
3.	Study objectives .....	6
3.1	Overall objectives .....	6
3.2	Specific objectives .....	6
4.	Study implementation .....	6
4.1	WHO Performance Evaluation Laboratories .....	6
4.2	Training, performance evaluation and supervision .....	6
4.3	Safety .....	7
4.4	Storage of assays .....	7
5.	Specimens .....	8
5.1	General description of the panel .....	8
5.2	Specimen collection and storage .....	8
5.3	Characterization of the cholera specimen panel .....	8
5.4	Data collection .....	9
6.	Laboratory testing .....	10
6.1	Review of instructions for use .....	10
6.2	Sequence of testing .....	10
6.3	Reading test results .....	10
6.4	Recording test results .....	11
7.	Quality control .....	11
7.1	Competency panels .....	11
7.2	Internal control lines for rapid diagnostic tests .....	11
7.3	Test kit controls .....	12
7.4	External quality control specimen .....	12
7.5	QC acceptability .....	12
8.	Analysis of data .....	12
8.1	Invalid test devices .....	12
8.2	Clinical performance characteristics .....	12
8.2.1	Calculation of sensitivity and specificity .....	13
8.2.2	<i>V. cholerae</i> O1/O139 discriminatory tests .....	13
8.2.3	Discrepant results .....	13
8.2.4	Proportion of results with very weak lines .....	13
8.3	Inter-reader variability .....	14
8.4	Inter-lot variability .....	14
9.	Technician's appraisal .....	14
10.	Ethical considerations .....	14
10.1	Compliance with International Standards .....	14
10.2	Specimen collection .....	14
10.2.1	Use of left-over specimens .....	14
10.2.2	Purposeful collection of new specimens .....	14
10.3	Informed Consent .....	15
10.3.1	Left-over specimens from routine surveillance .....	15
10.3.2	Left-over specimens from research projects .....	15
10.3.3	Purposeful collection of new specimens .....	16
10.4	Risk-Benefit assessment .....	16
10.5	Storage of data and specimens .....	16
10.5.1	Confidentiality .....	16

10.5.2	Data storage & biobanking.....	16
10.6	Results and incidental findings policy.....	16
11.	Report preparation and dissemination .....	17
12.	Materials and supplies.....	18
13.	Roles and responsibilities .....	18
13.1	Responsibilities of the PEL.....	18
13.2	Responsibilities of WHO .....	18
13.3	Responsibilities of the manufacturer .....	18
14.	Other documents required .....	19
15.	References .....	19
16.	Document revision history.....	19
17.	Annexes .....	21
17.1	Annex 1. Operational characteristics and ease of use assessment form .....	21
17.2	Annex 2. Information Consent form.....	25
17.3	Annex 3. Information Sheet for minor (7-17 years of age).....	28

## 1. Introduction

### 1.1 WHO Prequalification of In Vitro Diagnostics

World Health Organization (WHO) prequalification of in vitro diagnostics (IVDs) is coordinated through the department of Regulation and Prequalification. Focus is placed on IVDs for priority diseases and their suitability for use in resource-limited settings.

WHO prequalification of IVDs is a comprehensive quality assessment of individual IVDs through a standardized procedure aimed at determining whether the product meets WHO prequalification requirements (1). Two types of prequalification assessment can take place, depending on the regulatory version submitted and evidence from a previous stringent review by a Recognized Stringent Regulatory Authority.

The full prequalification assessment process includes the following components:

- review of a product dossier
- performance evaluation including operational characteristics
- inspection of the manufacturing site(s)
- labelling review.

Performance evaluations for WHO prequalification assessment are conducted by a Performance Evaluation Laboratory (PEL) following a choice of two different mechanisms described [here](#)<sup>1</sup>. Performance evaluations under option 1 will be conducted by a laboratory in List 1 and coordinated and cost covered by WHO. Performance evaluations under option 2 will be conducted by a laboratory in List 2 and coordinated and cost incurred by the manufacturer.

### 1.2 WHO performance evaluation of rapid diagnostic tests for the detection of *Vibrio cholerae* O1 or O1/O139

This protocol is a master protocol that describes the procedures required to perform the evaluation of rapid diagnostic tests (RDTs) for the detection of *Vibrio cholerae* O1 or O1/O139 submitted for WHO prequalification assessment. **This protocol is not intended to replace validation and verification studies that need to be conducted by the manufacturer in order to fulfil WHO prequalification product dossier requirements.**

The performance evaluation determines the accuracy of rapid diagnostic tests for detection of *V. cholerae* O1 or O1/O139 in comparison with established reference methods. The evaluation characteristics include clinical performance (sensitivity, specificity) determined in a laboratory environment. In addition, operational characteristics and ease of use are assessed to inform use in settings with limited infrastructure.

Given the variety of assays available, this protocol remains generic in nature and some sections may be open to interpretation. Manufacturers are encouraged to contact WHO before the start of the evaluation for any question related to the applicability of this protocol to their specific assay.

## 2 Intended audience

This document is intended to provide PEL and IVD manufacturers with the WHO performance evaluation procedure for prequalification assessment.

---

<sup>1</sup> <https://extranet.who.int/prequal/vitro-diagnostics/performance-evaluation>

*Protocol for the performance evaluation of rapid diagnostic tests for the detection of *Vibrio cholerae* O1 or O1/O139*

IVD/PR/4/P18 (PQDx\_305) v. 2.2, November 2023

This protocol is NOT intended to be a master or guidance protocol for analytical and/or clinical validation and verification studies conducted by manufacturers for their product dossier or any other stakeholder.

### 3 Study objectives

#### 3.1 Overall objectives

The overall objective is to verify the performance of RDTs for detection of *V. cholerae* O1 or O1/O139 submitted to WHO prequalification assessment.

#### 3.2 Specific objectives

The specific objectives of the evaluation are:

- to determine the sensitivity and specificity of the RDT under evaluation for the detection of *V. cholerae* O1 or O1/O139 as compared to reference methods including bacterial culture and detection of *V. cholerae* specific DNA sequences encoding genes of interest using polymerase chain reaction (PCR);
- to estimate inter-reader and inter-lot variability;
- to describe and assess the operational characteristics and ease of use of the RDT under evaluation and its suitability for use in countries with limited infrastructure (no/limited electricity, no/limited clean water, and inadequate means of biosafety disposal, limited skills).

### 4 Study implementation

#### 4.1 WHO Performance Evaluation Laboratories

The PEL shall be one that has undergone assessment using the WHO Alternative Laboratory Evaluation Mechanism which includes submission of an expression of interest (EoI), Stage 1 audit (assessment of EoI and specific quality management system (QMS) documentation), Stage 2 on-site audit to assess compliance with WHO requirements. The list of PEL can be found using this [link](#)<sup>2</sup>.

The laboratory shall hold the following certification for quality management within the laboratory: ISO17025 (General requirements for the competence of testing and calibration laboratories), ISO15189 (Medical laboratories: Particular requirements for quality and competence) or equivalent.

The person(s) listed in the EoI letter to WHO will act as the Principal Investigator (PI) and primary contact for the work performed by the PEL.

This evaluation may be conducted simultaneously in more than one PEL, to ensure that the total sample size can be reached together.

#### 4.2 Training, performance evaluation and supervision

The following issues are key to minimize errors and maximize the value of this evaluation.

- The PI will be responsible for training the laboratory staff on the evaluation protocol and on testing of each assay undergoing evaluation.

---

<sup>2</sup> <https://extranet.who.int/prequal/vitro-diagnostics/prequalified/performance-evaluation-laboratories>  
*Protocol for the performance evaluation of rapid diagnostic tests for the detection of Vibrio cholerae O1 or O1/O139*  
IVD/PR/4/P18 (PQDx\_305) v. 2.2, November 2023

- Only those laboratory staff who have received specific assay training for the evaluation will be involved in the assessment.
- Accurate record keeping is crucial to the success of the evaluation and the PI will be responsible for ensuring that all data required for the evaluation are recorded on the agreed data collection sheets, and they are accurate, legible and up to date and cross checked.
- It is important to plan work in advance and follow standard operating procedures as prepared and controlled by the PEL.
- To reduce the risk of adding an incorrect specimen to a test device or well, before starting the test run, the operator will prepare worksheets and label all tubes, dilution vessels, test devices with the specimen's unique number.
- Because objective, machine-generated, permanent results for subjectively read tests are not feasible, it is essential that the PI emphasizes to the operators performing the tests the need for accurate recording of results and record keeping.
- To minimize the risk of error and assess inter-user agreement, the results are read and recorded independently by three trained staff members.
- To allow immediate correction of erroneous recording of results (rather than differences in visual interpretation), the PI or designee should review the results within the range of time recommended by the manufacturer to allow him/her to return to the original test device to investigate apparently discordant readings.
- For subjectively-read assays, electronic images of at least one positive, one negative, as well as all discrepant, invalid or otherwise unexpected results (e.g. anomalies) will be documented and recorded.

### **4.3 Safety**

Cholera and other potential pathogens found in stool specimens are transmissible by contact. Therefore, all specimens must be handled as potentially infectious. Appropriate precautions to minimize infectious hazards must be taken at all stages from the collection of specimens to the disposal of used materials from the laboratory. The Laboratory biosafety manual (2) and the evaluating site's guidelines on laboratory safety shall be strictly adhered to by the laboratory staff involved in such activities.

### **4.4 Storage of assays**

All reagents shall be stored as indicated in the assay instructions for use. Calibrated thermometers or other environmental monitoring devices will be placed at each location where reagents and specimens will be stored, i.e. ambient, refrigerator and freezer. Temperatures will be recorded daily. The lot numbers of the test kits received and used and their expiry dates will be recorded on the individual run worksheets.

Two separate production lots (with different lot numbers and different expiry dates) will be requested for the performance evaluation, according to the following definition of a lot<sup>3</sup>: "The amount of material that is uniform in its properties and has been produced in one process or series of processes. The material can be either starting material, intermediate material or finished product." Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents. Furthermore, lots must be sourced

---

<sup>3</sup> ISO 18113-1:2022 In vitro diagnostic medical devices -Information supplied by the manufacturer (labelling) – Part 1: Terms, definitions and general requirements

from a representative production run and not produced especially for the purpose of this evaluation. WHO will verify this information before the product assessment has been finalized.

## 5 Specimens

### 5.1 General description of the panel

The panel will consist of fresh stool specimens collected from adult and children (>5 years of age) patients with watery diarrhoea and suspected to have cholera infection. The panel will comprise a minimum of **120 confirmed positive specimens** and **200 confirmed negative specimens**, as characterized by the algorithm described below (section 5.3).

This sample size will ensure a precision (total width of the confidence interval using the binomial exact calculation) of 12% around a sensitivity of 90% and of 7% around a specificity of 95%, as described in the target product profile for the development of improved cholera rapid diagnostic tests (3) or 10% around a specificity of 85%, set as minimal performance in the Interim Technical note on the use of cholera rapid diagnostics tests (4).

### 5.2 Specimen collection and storage

Specimens will be prospectively collected from adults and children (>5 years of age) patients with watery diarrhoea and suspected to have cholera infection.

The panel may consist of the following specimens:

- left over specimens from routine surveillance activities
- left over specimens from studies
- specimens collected purposefully for this evaluation.

The specimens will be freshly collected in appropriate containers or using rectal swabs depending on the manufacturer's recommendations described in the assay's instructions for use (IFU). The specimen handling, transport and storage conditions described in the IFU will be followed. In particular, processing of the specimen shall happen in a timely manner respecting the manufacturer's claim for specimen stability.

All personal identifiers will be removed, and specimens will be given a unique identification number.

Ethical considerations are described in section 10.

### 5.3 Characterization of the cholera specimen panel

The freshly collected stool specimens that will be used in the performance evaluation shall be transported and stored according to the manufacturer's instructions and then characterized using a standardized combination of assays (testing algorithm). These results will be used to determine the cholera status of each specimen for the purpose of the performance evaluation (see Figure 1). Use of any other combination of assays for characterization of the cholera specimen evaluation panel shall be communicated, discussed and agreed with WHO beforehand.

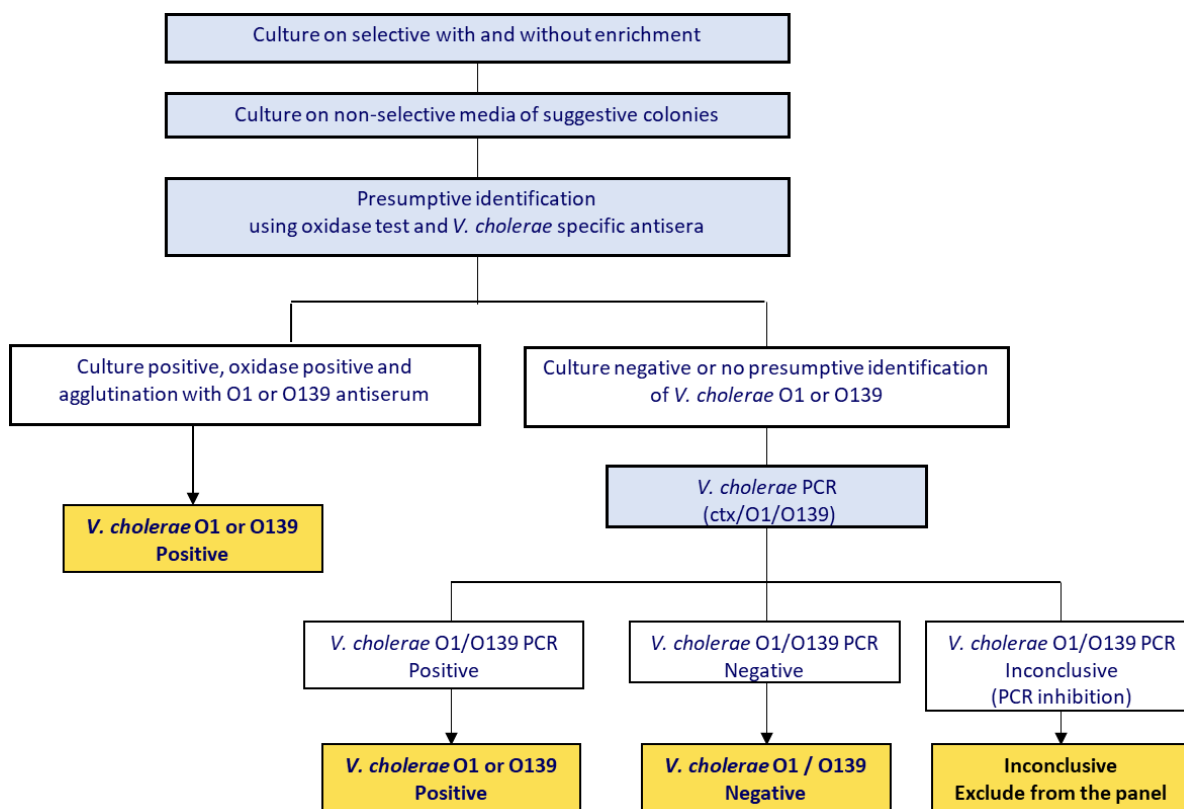
Initially, each specimen will be inoculated into an enrichment medium (alkaline peptone water for 6 hours at  $35\pm 2^{\circ}\text{C}$ ) and directly onto a selective media plates (Thiosulfate Citrate Bile-Salts Sucrose (TCBS)). The enriched medium will also be inoculated onto a selective media plate. After overnight incubation of the plates at  $35\pm 2^{\circ}\text{C}$ , the plates will be observed for the presence of colonies suggestive of *V. cholerae*. Phenotypically characteristic colonies will then be sub-cultured on a non-selective medium (i.e. Mueller Hinton (recommended), Brain Heart Infusion Agar, or Trypticase Soy Agar) and incubated at  $35\pm 2^{\circ}\text{C}$  overnight. This



culture on non-selective medium will allow to perform the oxidase test and to determine the serogroup by using specific antisera to differentiate *V. cholerae* O1 and O139 from other enteric pathogens.

Specimens giving colonies with a characteristic *V. cholerae* phenotype, oxidase positive and agglutinating with specific *V. cholerae* O1 or O139 antisera will be considered positive for *V. cholerae* O1 or O139.

Specimens that are negative for *V. cholerae* O1/O139 by culture will be tested using a commercialized PCR kits for the detection of *V. cholerae* O1 and O139 validated for the use on human stool specimens. Specimens that are culture and PCR negative for *V. cholerae* will be considered as confirmed negative for *V. cholerae* O1 and O139. Specimens that are culture negative but positive for *V. cholerae* by PCR will be considered as positive for *V. cholerae* O1 or O139. Specimens that are culture negative and with an inconclusive PCR result (PCR inhibition suspected) will be considered as inconclusive and excluded from the panel.



**Figure 1. Testing algorithm for the characterization of the specimen panel**

Note: For the evaluation of tests for the detection of *V. cholerae* O1 only, specimens confirmed positive for O139 by culture and/or PCR will be considered as negative for the analysis.

#### 5.4 Data collection

The date of specimen collection will be recorded.

In addition, patient’s age and sex may be collected, if available, either through patient interview (for purposeful collection of specimens) or through patient hospital admission file.

## 6 Laboratory testing

### 6.1 Review of instructions for use

Each product under evaluation is used strictly in accordance with the instructions for use (IFU) issued by the manufacturer. The PEL will send a copy of the IFU to WHO/PQT upon delivery of the test kits and prior to commencement of the laboratory evaluation. The IFU shall be reviewed against the IFU submitted to WHO/PQT as part of the dossier assessment for the prequalification assessment. **If the IFU has been updated since dossier submission, a letter detailing the changes made shall be sent to WHO/PQT before the laboratory evaluation commences.**

### 6.2 Sequence of testing

The WHO cholera specimen panel will be run in parallel on two lots. The specimens will be tested on the assay under evaluation **in a blinded manner**, i.e. the readers will not be aware of the reference result or of reading of other readers.

Specimens with invalid results should be retested once on the same lot.

Specimens with results discrepant from the reference result will be retested once on each lot by the same operator.

Whenever possible, repeat testing should be done on stools stored at 4°C, within the recommended time for specimen processing. Otherwise, specimens may be stored at -20°C for future testing, if in line with the manufacturer's recommendations.

- If the repeat testing results are conflicting with the initial results (i.e. non-reactive versus medium to strong reactive, see Table 1 below) and an operator error cannot be excluded, then the result for this specimen will be excluded from the analysis.
- If the repeat testing results are not conflicting with the initial results (i.e. same result or negative versus very weak or weak result, see Table 1 below), then the initial result will be used for the analysis. The results of repeat testing will be described, but not used in analysis of sensitivity or specificity.

In all cases of repeat testing, all results (initial and repeat testing) should be recorded.

### 6.3 Reading test results

The interpretation of results of the assay under evaluation is made strictly according to the manufacturers' IFU.

Visual interpretation of results of subjectively read assays is made independently by three readers, without the knowledge of the other two sets of results and blinded to the reference result for the specimen. In addition, each reader grades the intensity of line/spot, as described in Table 1.

**Table 1. Result legend for subjectively read assays**

Scoring index	Intensity reading scale
0	Non-reactive
1	Very Weak
2	Weak
3	Medium to Strong Reactivity

If the three readers interpret the results differently from each other, the consensus is recorded as that interpretation (reactive vs non-reactive) which occurs two out of three times.

In addition, for reactive results, when at least two readers graded the line as very weak, the result will be reported as very weak reactive.

Finally, the operator will also report any anomaly with the device, according to Table 2.

**Table 2. Type of anomalies recorded in the evaluation and corresponding codes**

Code	Description
IC	Incomplete clearing
MI	Incomplete migration
FM	Failed migration
SM	Strip misplaced in cassette (shift)
PAD	Specimen pad not seen in sample window
GL	Ghost test lines
DL	Diffuse test lines
PL	Patchy broken test line
BP	Buffer remains pooled in buffer well
OT	Any other anomaly

#### 6.4 Recording test results

All test results are recorded on standardized worksheets and then entered in a Microsoft Excel spreadsheet (see Section 14. Other documents required) for further data analysis.

Invalid results and indeterminate results, if applicable, are also recorded in the data collection sheets, together with the repeat testing results.

After all results are recorded, they are verified by the operator so that any mistakes may be identified and rectified immediately. Should recording errors be identified, both the original and corrected results are recorded and initialled by the operator. In addition, for results on the clinical panel, the operator compares the results of the assay under evaluation with the reference results, to identify specimens with discrepant results, which need to be repeated.

Prior to analyses, the databases will be checked, either by comparison of double data entry (with Excel Pro) or by comparing the printout of the Excel spreadsheet with laboratory worksheets.

## 7 Quality control

### 7.1 Competency panels

Before the start of the evaluation, a competency panel must be run successfully for each assay by each operator. This will be done by testing a panel of 10 well-characterized frozen stool specimens, including cholera positive and negative specimens.

### 7.2 Internal control lines for rapid diagnostic tests

Generally, rapid diagnostic tests contain a control band, line or spot to determine that the test device is operating correctly, and the result is valid. In absence of the control band the result is read as invalid. Most

*Protocol for the performance evaluation of rapid diagnostic tests for the detection of Vibrio cholerae O1 or O1/O139*

control bands, lines or spots will become visible with the addition of reagent (i.e. buffer) only. However, some rapid diagnostic tests may contain a control band, line or spot that also controls for the presence of the specimen (i.e. addition of stool specimen). It is imperative that the exact nature of the control band, line or spot is ascertained and recorded in the report. A test run is performed to verify this point before the evaluation starts, if not explicitly mentioned in the IFU.

### **7.3 Test kit controls**

If available, manufacturer-supplied positive and negative test kit controls will be run as indicated in the IFU at the commencement of each testing session for RDTs (i.e. every day, and for each lot used). Where positive and negative test kit controls are not supplied by the manufacturer, as will be the case for many rapid diagnostic tests, external quality control specimens (cholera positive and negative specimens) will act as the control specimens, see section 7.4.

### **7.4 External quality control specimen**

The PEL supplies an external quality control (QC) specimen which is tested singly at the beginning of each test session for RDTs. The QC specimen represents a weakly reactive specimen (i.e. close to the limit of detection of the test), and thus may be different for different assays and different assay formats.

### **7.5 QC acceptability**

All results of test kit controls and external QC specimens will be entered into the data collection sheets. Should the test kit controls or the QC specimen not give results within the expected ranges, the evaluation process of that assay will be suspended until the cause has been identified and a satisfactory solution is identified. Such problems must be communicated immediately to WHO and must be recorded on the data sheets. The PI will be responsible for carefully checking all data entry forms for legibility, accuracy and completeness.

## **8 Analysis of data**

### **8.1 Invalid test devices**

The number of invalid results is recorded and presented as a percentage of the total number of tests used for the clinical evaluation.

Invalid results may mean invalid test results as defined by the instructions for use such as where the control line/spot does not appear or invalid due to obviously defective test device or defective transfer pipette.

### **8.2 Clinical performance characteristics**

The following method will be used to calculate the performance characteristics by comparing the results of each lot of the assay under evaluation with that of the reference on the clinical specimen panel. Data will be presented in 2x2 table(s) describing the results of the assay under evaluation compared to the results of reference testing, as shown in Table 3.

For specimens giving a first invalid result and a valid repeat result, the valid result will be used for the analysis. Specimens giving repeated invalid results will be excluded from this analysis.

**Table 3. 2 x 2 table for calculation of performance characteristics**

		Results of reference testing		
		+	–	Total
Results of assay under evaluation	Reactive	a True positives	b False positives	a+b
	Non-reactive	c False negatives	d True negatives	c+d
	Total	a+c	b+d	

### 8.2.1 Calculation of sensitivity and specificity

Sensitivity is the ability of the assay under evaluation to correctly detect specimens that contain *V. cholerae* O1 or O139 (reference results positive). Thus, sensitivity is the number of true positive specimens identified by the assay under evaluation as reactive (a), divided by the number of specimens identified by the reference algorithm as positive (a+c), expressed as a percentage.

$$\text{Sensitivity} = \frac{a}{a + c}$$

Specificity is the ability of the assay under evaluation to correctly detect specimens that do not contain cholera antigen (reference results negative). Thus, specificity is the number of true negative specimens identified by the assay under evaluation as non-reactive (d), divided by the number of specimens identified by the reference assays as negative (b+d), expressed as a percentage.

$$\text{Specificity} = \frac{d}{b + d}$$

Sensitivity and specificity will be reported with their exact 95% confidence intervals for binomial proportions.

### 8.2.2 *V. cholerae* O1/O139 discriminatory tests

For tests that can discriminate *V. cholerae* O1 and O139, the main analysis of sensitivity and specificity will be performed globally, as described above (i.e. for *V. cholerae* O1 and O139 combined). In addition, the results for each line will be reported and the proportion of specimens for which the *V. cholerae* serogroup was correctly identified will be calculated. False positive results will also be described for each line.

### 8.2.3 Discrepant results

As described in section 5.2, specimens with results that are discrepant from the reference result are retested once on each lot.

Results of repeat testing will be described but will not be used in calculations of sensitivity and specificity. However, if the results of repeated testing are in conflict with initial result and an operator error cannot be excluded, then the specimen will be excluded from the analysis (see section 6.2).

### 8.2.4 Proportion of results with very weak lines

The proportion of initial results with very weak line among reactive results will be described globally and among positive and negative specimens by the reference assay(s). This will be done separately for the O1 and O139 lines, if applicable.

### **8.3 Inter-reader variability**

Inter-reader variability is calculated when assay reading is performed without objective reading instruments i.e. RDTs. Three persons independently interpret each test result. The inter-reader variability is expressed as the percentage of specimen for which initial test results are differently interpreted (i.e. reactive or non-reactive) by the independent readers.

Inter-reader variability is assessed for each test line/dot if applicable.

### **8.4 Inter-lot variability**

Inter-lot variability will be assessed by comparing the results of the clinical specimen on each lot. Results will be presented in a 2x2 table. The proportion of discrepant results will be reported. In addition, a McNemar test for paired data will be applied, and agreement will be estimated using a kappa coefficient.

## **9 Technician's appraisal**

The technical aspects of the assay under evaluation will be assessed by the technician who performed the testing using a standardized form (Annex 1). These assessments, along with other selected assay characteristics, contribute to an overall appraisal of each assay's suitability for use in small laboratories.

## **10 Ethical considerations**

### **10.1 Compliance with International Standards**

This protocol was submitted to the World Health Organization (WHO) Ethical Review Committee and was approved (ERC.0003781). When required by local regulations, this protocol will be submitted to the national and institutional ethical review boards of the PEL. Site specific ethics approval should be acquired as per local Institutional Review Board requirements. Any substantial change to the protocol must be approved by all the bodies that have approved the initial protocol, prior to being implemented, unless it is due to participant's safety concerns. The evaluation will be carried out according to the principles stated in the Declaration of Helsinki as amended in 2013 and any further updates, all applicable national and international regulations.

### **10.2 Specimen collection**

This evaluation will require clinical specimens belonging to different categories. As stated in section 4, these categories include:

#### *10.2.1 Use of left-over specimens*

Residual specimens from routine surveillance may be used in the evaluation. If applicable according to local regulations, participants are made aware of the potential secondary use of their specimens through written informed consent prior to collection. Alternatively, informed consent can be waived by local ethics committees when specific conditions are met (see 10.3.1). In some cases, left-over specimens from research studies can be used, under the conditions stated in 10.3.2.

#### *10.2.2 Purposeful collection of new specimens*

Specimens may be collected from participants, specifically for the purpose of this evaluation (i.e. not as part of ongoing routine clinical practice). Participants will be asked to provide written informed consent before collection of the specimen.

## 10.3 Informed Consent

### 10.3.1 *Left-over specimens from routine surveillance*

When using left-over specimens from routine care for non-research purposes, the need for written informed consent can be waived by local ethical review boards, provided one of the following conditions are met. If these conditions are not met, or if specimens are collected in countries where such waivers do not apply, only specimens with written informed consent for secondary use of specimens, as described above, will be used.

#### Presumed consent

In some countries, the need for written informed consent for secondary use of left-over specimens from routine care may be waived based on presumed consent. In this case, prior to collection, specimen donors are made aware of the potential secondary use of their specimen for research purposes. This may be done through several channels, including: clearly visible pamphlets and posters at the collection site, available information on the institute's website and personal communication through the treating physician or nurse. The participant will be made aware of the right to refuse and opt out without any consequence for the quality of care.

#### Anonymization

It is possible in some countries to obtain a waiver for local ethical review when using fully anonymized left-over samples from routine practice for non-research purposes. It is the responsibility of the PEL to check the requirements for ethical and regulatory approval with their own institution.

### 10.3.2 *Left-over specimens from research projects*

Left-over specimens from research studies may be used if written informed consent on secondary use of the specimens has been acquired from participants prior to collecting the specimen. As per good ethical practice, it is advisable that informed consent forms used to obtain consent for secondary use contains at least the following information in clear understandable language respective to the target audience: *the possibility of specimen storage and secondary use for specified purposes; that participation is voluntary and the participant is free to withdraw without consequences; what (if any) compensation the participant will receive and any other benefits related to the participant; any foreseeable risks involved in participating; provisions made to respect and preserve the participants privacy and confidentiality; that the protocol/ICF was reviewed by relevant ethical bodies.*

If consent for secondary use has not been obtained, but the following conditions are met, de-identified left-over specimens from research studies can also be used in this evaluation if:

- a. the study informed consent does not specify that the specimens will only be used for this specific study;
- b. and the originally indicated timeframe for sample destruction in the informed consent is respected;
- c. and/or\* the local regulations allow for such use.

\*In case "b" restricts the use of the sample for this purpose, it may be argued that the public health benefit of the purpose outweighs the harm that could be caused by having to re-consent all participants and re-consenting might consequently be waived for use of the specimens for this evaluation.

### *10.3.3 Purposeful collection of new specimens*

Where purposeful collection of new specimens is used, written informed consent will be acquired from participants prior to collecting the specimen (see Annex 2). The participant will be provided with all necessary information on the purpose of the evaluation, both on paper and by staff. For participants aged 5 to 17 years, written informed consent will be obtained by the parent and legal guardian and oral assent will be obtained from the minor participant (Annex 3). The information forms in Annex 2 and Annex 3 will be translated into local language. In case of illiteracy, the information will be read to the participant and a fingerprint will be used as a signature. Two copies of the informed consent form will be completed, one will be kept by the staff at the evaluating laboratory and one by the participant.

### **10.4 Risk-Benefit assessment**

There will be no direct benefits to the specimen donors. PELs may decide to provide a modest degree of compensation (e.g. to cover expenses for transport), without leading to undue inducement. The results obtained in this evaluation will be used as part of WHO prequalification assessment, to ensure that the tests meet WHO requirements. There will thus be broader benefits to communities affected by cholera by contributing to the selection of well-performing cholera rapid diagnostic tests.

There is no risk associated with stool specimen collection. Risks of breach of confidentiality will be minimized by collecting as little personal information as is necessary and ensuring proper data protection according to general data protection regulations as well as anonymization of specimens and data as soon as a link to identifiable information is no longer required.

### **10.5 Storage of data and specimens**

#### *10.5.1 Confidentiality*

Alongside collection of the specimen, some personal data may be recorded. This includes age, gender, and collection date. All non-essential personal identifiers (including direct identifiers such as name, address, etc.) will be removed and specimens will receive a unique identification number at the collection site. In short, all collected data will be pseudonymized and transformed in order to preserve participant's privacy. When a link with the original identifiable information is no longer required, this link will be destroyed, and data will be anonymized.

#### *10.5.2 Data storage & biobanking*

Personal data will be handled and stored according to the European general data protection regulations (GDPR) or local alternatives where applicable. Any documents containing the names and/or signatures of participants (e.g. consent forms) will be kept separately from all other evaluation documents containing participant data. All evaluation documents will be stored in lockable rooms or cabinets with access limited to evaluation staff. Names of the participants will not appear on any reports or publications resulting from this evaluation.

After the finalization of the evaluation report, all source data, data analysis records and all correspondence will be retained at the testing laboratory for five years under the PI's custody. Specimens may be stored according to local policy.

### **10.6 Results and incidental findings policy**

The evaluation will not interfere with the clinical care the participant would normally receive, if applicable. None of the results generated in this evaluation from evaluated assays will be used as a replacement for the



gold-standard tests currently in use. As the purpose is to evaluate new assays, incidental findings are unlikely to occur. Should such a finding of significant clinical importance occur, the subsequent actions (e.g. feedback to the patient) shall be considered on a case-by-case basis by the evaluating laboratory and its medical staff according to their incidental findings policy.

## 11 Report preparation and dissemination

The preliminary data analysis and drafting of the report will be carried out by the PEL according to pre-defined report templates (see Section 14, Other documents required).

For evaluations coordinated by WHO (option 1), the draft report will be shared with WHO. WHO will verify the data analysis and draft report and send the final draft report to the authorized contact designated by the manufacturer for comment.

For evaluations commissioned by the manufacturer (option 2), the data and draft report will be shared simultaneously with WHO and the manufacturer in copy. WHO will verify data analysis and draft report and produce a final draft, which will be shared with the PEL and the manufacturer.

In both cases, manufacturers will have one month right of reply after WHO has officially shared the draft report. After one month has elapsed, the report will be accepted as final by WHO, regardless if comments are submitted. The final report will be prepared and disseminated by WHO. A copy of the final report will be sent to the authorized contact designated by the manufacturer and to the evaluating laboratory.

If the assay under evaluation successfully meets all WHO prequalification requirements, a summary of these data will be published in the WHO Public Report for the prequalification assessment of the assay.

The PEL(s) conducting the evaluation will inform local and national authorities of the evaluation through seminar/dissemination and will share the WHO Public Report in case of positive outcome of the prequalification assessment.

WHO reserves the right to publish the results of the evaluation, regardless of the outcome. In this case, WHO will share the manuscript with the manufacturer for comments at least 30 days before submission, but WHO will have ultimate authority over the version submitted. Authors will include contributors from WHO and the PEL.

Any publication by WHO of the results of these evaluations and the WHO recommendations derived therefrom will, however, be accompanied by the following disclaimer:

*The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.*

*WHO and the Performance Evaluation Laboratory, do not warrant or represent that the evaluations conducted with the test kits referred to in this document are accurate, complete and/or error-free. WHO and the PEL disclaim all responsibility for any use made of the data contained herein, and shall not be liable for any damages incurred as a result of its use. This document must not be used in conjunction with commercial or promotional purposes.*

## 12 Materials and supplies

The manufacturers will provide the products and any equipment necessary for the evaluation free of charge. Two different lots of tests will be needed.

**Table 4. Number of tests required to perform this evaluation**

	Number of tests
Training, competency testing	50 per site
Clinical specimen panel	~500 on each lot depending on prevalence
<b>Total + ~10% for controls and repeats</b>	<b>1200</b>

## 13 Roles and responsibilities

### 13.1 Responsibilities of the PEL

- i. Ensure availability of specimens, including competency panel;
- ii. Submission of the protocol to local/national ethics committee if required;
- iii. Conducting the performance evaluation in accordance with the protocol and with internationally recognized best practice;
- iv. Data analysis and preparation of draft report of the performance evaluation and sharing data and draft report with WHO (and manufacturer for option 2 evaluations);
- v. Advising WHO on operational characteristics of assays evaluated;
- vi. Archiving all source data, data analysis records and all correspondence for a period of at least ten years.

### 13.2 Responsibilities of WHO

- i. Technical and administrative management of the performance evaluation (evaluations under option 1);
- ii. Technical advice to the PI;
- iii. Verification of analysis and draft report and, if the evaluation was conducted in more than one PEL, compiling the final draft report;
- iv. Communication of the final draft report to manufacturer and seeking of comments from manufacturer;
- v. Preparation and dissemination of the final report;
- vi. Formal contacts with the manufacturers.

### 13.3 Responsibilities of the manufacturer

- i. Providing the appropriate number of test free-of-charge for the evaluation;
- ii. Ensuring that kits are shipped under appropriate conditions and in time for the commencement of the evaluation;
- iii. Providing comments to WHO on the draft performance evaluation report within one month;
- iv. For option 2 evaluations, selection of the PEL, agreement on terms and conditions of the evaluations in line with the conditions set forth in the Letter of Agreement with WHO, and funding of the evaluation process.

## 14 Other documents required

### Master Templates

IVD/TP/4/P18a PQDx\_370 Template report for performance evaluation of cholera rapid diagnostic tests

IVD/TP/4/P18b PQDx\_311 Template data entry spreadsheet for the performance evaluation of cholera rapid diagnostic tests

## 15 References

1. World Health Organization. Overview of the WHO Prequalification of In Vitro Diagnostics Assessment. Version 9. [Internet]. Geneva: World Health Organization; 2021. Available from: <https://extranet.who.int/pqweb/key-resources/documents/overview-who-prequalification-vitro-diagnostics-assessment>
2. World Health Organization. Laboratory biosafety manual, 4<sup>th</sup> edition [Internet]. Geneva: World Health Organization; 2020. Available from: <https://www.who.int/publications/i/item/9789240011311>
3. Global Task Force on Cholera Control. Target Product Profile (TPP) for the development of improved Cholera rapid diagnostic tests [Internet]. 2017 [cited 2021 Mar 8]. Available from: <https://www.gtfcc.org/wp-content/uploads/2019/10/gtfcc-tpp-for-the-development-of-improved-cholera-rapid-diagnostic-test.pdf>
4. World Health Organisation. Interim Technical Note The Use of Cholera Rapid Diagnostic Tests. 2016;(November):1–5. Available from: <https://www.gtfcc.org/wp-content/uploads/2019/10/gtfcc-interim-use-of-cholera-rapid-diagnostic-tests.pdf>

## 16 Document revision history

Protocol version	Effective date	Revisions	Prepared by
1.0	March 2017	Initial release	Willy Urassa
2.0	9 May 2022	Editing (changed organization of sections); specified that evaluation could be done in 2 sites simultaneously; changed sample size (from 200 to 120 cholera positive specimen; from 500 to 200 negative specimens); changed PCR test used in the characterization algorithm due to unavailability of test included previously; changed to testing clinical panel in parallel on 2 lots and analysing inter-lot variability; changed grading scale (added “weak”, in addition to “very weak” and “medium to strong”) and added that proportion of very weak lines will be reported; specified how repeated results for discrepant specimens will be used; added section on ethics considerations; updated the operational characteristics and ease of use forms	AL Page  External review by: Marie-Laure Quilici, Institut Pasteur, France; Amanda Debes, Johns Hopkins Bloomberg School of Public Health
2.1	13 September 2022	Requests for clarification from WHO ERC; minor editing	AL Page

*Protocol for the performance evaluation of rapid diagnostic tests for the detection of Vibrio cholerae O1 or O1/O139*

IVD/PR/4/P18 (PQDx\_305) v. 2.2, November 2023

2.2	27 October 2023	Addition of ISBN, copyright page and barcodes; update of links and references; minor editing. Addition of new IVD protocol number IVD/PR/4/P18	AL Page
-----	-----------------	---	---------

## 17 Annexes

### 17.1 Annex 1 - Operational characteristics and ease of use assessment form

Table A1.1. Operational characteristics

1 Assay characteristics			
1.1	Assay format		Cassette, Dipstick, Card, Other (specify)
1.2	Need to reconstitute reagents		Yes/No
1.3	Total number of steps* to perform the assay		<i>Each action required to obtain a result (excluding specimen collection, device preparation, e.g. opening the pouch and reading)</i>
1.3.1	Number of steps with specified time limits		
1.3.2	Number of steps requiring precision pipetting		
1.4	Time from start to completion for one test		minutes
1.5	Endpoint stability		<i>Interval between minimum and maximum reading times - in minutes</i>
1.6	Type of internal control		Reagent addition control, Specimen addition control
2 Specimen collection and storage			
2.1	Type of specimen collection device provided in the kit		Loop, Capillary tube, Inverted cup, Pipette, No specimen collection device, Other (specify)
2.2	Validated specimen types		
2.3	Specimen volume(s)		<i>Specify by specimen type if different (µL)</i>
3 Safety			
3.1	Strip exposed		

<b>4 Kit storage</b>		
4.1	Number of tests per kit	<i>For the kit used for the evaluation</i>
4.2	Kit dimension	<i>width / depth / height (cm)</i>
4.3	Recommended storage temperature	
4.4	Recommended storage humidity	
4.5	Stability of reagents after opening	
<b>5 Equipment and consumables</b>		
5.1	What are the general laboratory equipment required to perform the assay but not provided	<i>E.g. precision pipette, vortex</i>
5.2	What are the consumables required to perform the assay but not provided	<i>E.g. tips, bleach, ethanol</i>

\* Definition: each action required to obtain a result (excluding specimen collection, device preparation – opening the pouch), e.g. for RDTs: add specimen, add buffer (2 steps).

**Table A1.2. Ease of use**

		Not applicable	Strongly disagree	Disagree	Agree	Strongly agree
<b>1</b>	<b>Instruction for use</b>					
1.1	The IFU is clear*					
1.2	If applicable, pictures/diagrams are clear*					
1.3	The IFU contains all important information**					
1.4	Safety instructions are clear*					
<b>2</b>	<b>Kit packaging and labelling</b>					
2.1	Kit labelling is clear*					
2.2	Kit packaging material is of good quality					
2.3	All buffers were provided in sufficient quantities					
2.4	If applicable, kit controls were provided in sufficient quantities					
<b>3</b>	<b>Use of devices and assay procedure</b>					
3.1	If applicable, the lancet is safe and easy to use					
3.2	If applicable, collection device is easy to use					
3.3	The test procedure is easy to perform					
<b>4</b>	<b>Reading and interpretation</b>					
4.1	The test line(s) is(are) always clear and easy to read					
4.2	The control line is always visible and easy to read					
4.3	Interpretation of the test is clear and easy*					
<b>5</b>	<b>Overall appraisal</b>					
5.1	Overall, the test is easy to use					
5.2	The test can be used in a laboratory with limited facilities					
5.3	The test can be used in non-laboratory settings					

\* Please keep in mind intended users

\*\* Refer to TGS-5 Designing instructions for use for in vitro diagnostic medical devices

<https://apps.who.int/iris/bitstream/handle/10665/259737/WHO-EMP-RHT-PQT-TGS5-2017.05-eng.pdf;sequence=1>

If disagree or strongly disagree, report item number and describe

n°	Comment



## 17.2 Annex 2. Information Consent form

### Informed Consent

#### Performance evaluation of rapid diagnostic tests for the detection of toxigenic *Vibrio cholerae*

##### *To be used only in case of purposeful collection of stool specimens*

[Name of Principle Investigator]: <Insert name>

[Name of Organization]: <Insert name>

This Informed Consent Form has two parts:

1. Information Sheet (to share information about the evaluation with you)
2. Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form.

### **PART I: Information Sheet**

#### **Introduction**

I am ....., working for [Name of the hospital]. We are doing an evaluation of rapid tests for the diagnosis of cholera. I am going to give you information and invite you to participate in this evaluation. You do not have to say right now whether or not you are willing to participate in the evaluation. Before you decide, you can talk to anyone you feel comfortable with about the evaluation. There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask me, the study staff.

#### **Purpose of the evaluation**

Cholera outbreaks are important threats for public health. It is important to detect and confirm cholera cases as early as possible to start public health measures quickly. Rapid diagnostic tests for cholera have the potential to facilitate the detection of cholera, but their ability to detect cholera cases has to be verified before being approved by the World Health Organization (WHO). We aim to evaluate the performance of rapid diagnostic tests for cholera.

#### **Type of evaluation**

This evaluation process will involve comparing the results of the cholera rapid diagnostic test compared to results obtained using more established and accurate standard test methods that are currently used to detect cholera.

#### **Participant selection**

We are inviting all patients with acute watery diarrhea attending this hospital to participate in the evaluation to assess the performance of cholera rapid diagnostic tests. In total, more than 360 participants will be included in this evaluation.

#### **Voluntary participation**

Your participation in this evaluation is entirely voluntary. It is your choice whether to give a stool sample or not. Whether you choose to participate or not, all the medical services you receive at this hospital/clinic will continue and nothing will change. You may change your mind later and stop participating even if you agreed earlier.

### **Procedures and Protocol**

Some stool will be collected in a pot. This will happen once, and you will not be asked to come back for this evaluation.

The specimen will be sent to [name of the laboratory], where it will be used with the cholera rapid diagnostic test and to the standard established tests for the diagnosis of cholera.

### **Risks**

We do not expect that any harm will happen to you because of joining this study.

### **Benefits**

If you participate in this evaluation, you will not have immediate individual benefits, but your participation is likely to help WHO to identify suitable simple rapid tests for the diagnosis of cholera which can be used in the future in order to help the surveillance of cholera all over the world.

### **Reimbursements**

You will not be given any money or gifts to take part in this evaluation.

### **Confidentiality**

The information that will be collected from this evaluation will be kept confidential. Information about you that will be collected during the evaluation will be put away and no one but the evaluation team will be able to see it. Any information about you will have a number on it instead of your name. Only the evaluators will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone except [name] who will have access to the information, such as evaluation sponsors, etc.

### **Sharing the Results**

Once the evaluation is finished, a report will be written by [name of the laboratory] and WHO. You may contact the laboratory if you would like to know the results of the evaluation. Confidential information will not be shared in this report. We may also publish the results of this evaluation in scientific journals so that other interested people may learn from our research. Your name will not appear in this publication, which will be only about the overall results.

### **Right to Refuse**

You do not have to take part in this evaluation if you do not wish to do so and refusing to participate will not affect your treatment at this hospital/clinic in any way. You will still have all the benefits that you would otherwise have at this hospital/clinic.

### **Who to Contact**

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following: [<Insert name>]

This proposal has been reviewed and approved by the <Insert name>, which is a committee whose task it is to make sure that evaluation participants are protected from harm. If you wish to find about more about the IRB, contact <Insert name>

You can ask me any more questions about any part of the evaluation study, if you wish to. Do you have any questions?

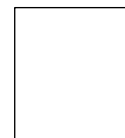
**PART II: Certificate of Consent**

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate /that my child participates in this evaluation.

Name of Participant \_\_\_\_\_ Date \_\_\_\_\_  
(Day/month/year)

Signature of Participant

OR Thumbprint



**If illiterate**

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness \_\_\_\_\_ Date \_\_\_\_\_ (Day/month/year)

Signature of witness

**Statement by the evaluation team leader/person taking consent**

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that we will collect some stool

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily. A copy of this ICF has been provided to the participant.

Name of Evaluation team lead/person taking the consent \_\_\_\_\_

Date \_\_\_\_\_ (Day/month/year)

Signature of Evaluator /person taking the consent

### **17.3 Annex 3. Information Sheet for minor (7-17 years of age)**

You are being hospitalized because you have diarrhoea. One possible cause for diarrhoea is cholera. It is important to detect cholera cases early to avoid outbreaks of cholera. The current methods for detecting cholera are complicated and take a long time. Rapid tests are available that could facilitate and shorten the detection of cholera cases. If these tests are performing well, they could be used here and in other countries to detect cholera cases and prevent outbreaks.

We are conducting an evaluation of a rapid diagnostic test for cholera and would like to ask you if you are willing to participate in this study. If you accept, we will take some stool and send it to the laboratory. There, your stool will be used to perform the rapid test and also the traditional method for the detection of cholera.

You don't have to participate. Nobody will be unfriendly or angry if you don't want to take part in this. You can always change your mind later on and say that you don't want to participate after all and you will not get in trouble for this. We will not let other people know your name. We cannot give you anything for your participation.

World Health Organization  
Avenue Appia 20  
1211 Geneva 27  
Switzerland  
[www.who.int](http://www.who.int)

9789240075047

