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Target product profile for a molecular test for **surveillance of cholera**

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Contents

Acknowledgements

The development of this target product profile (TPP) document was coordinated by FIND. The TPP development leadership team consisted of Jennifer Osborn (USA), Michael Messenger (UK), Wallace White (USA), Serafina Calarco (Switzerland) and Nadia Wauquier (WHO, Switzerland). The leadership team prepared the draft documents, facilitated TPP development group meetings and collated inputs from the TPP development group and the public throughout the TPP development process.

The authors are grateful to the individuals listed below who contributed to the preparation of this document.

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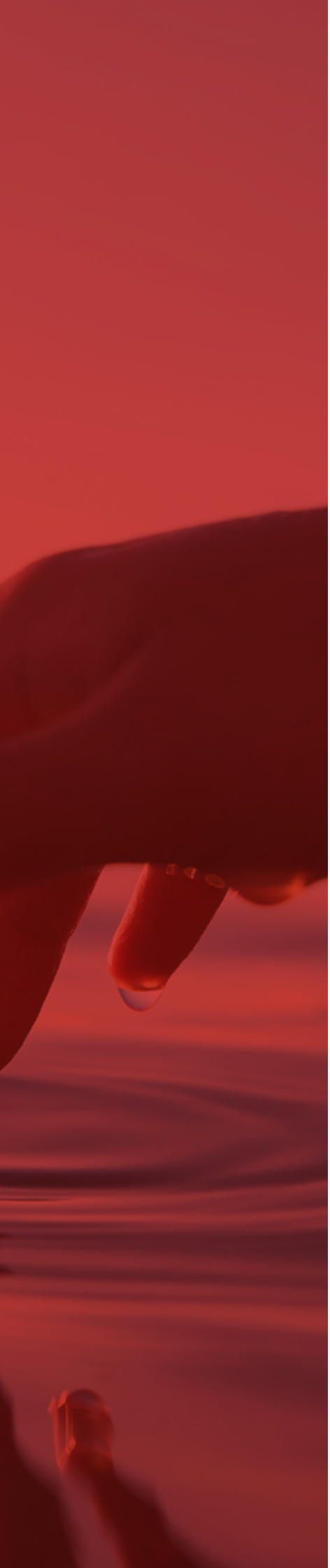

Declarations of interest

All members of the TPP development group completed a declaration of interest (DoI) form, according to FIND processes, that was used to assess and manage any conflicts of interest. FIND staff also checked there were no sanctions against any of the external members and conducted Google, LinkedIn and PubMed searches to identify any additional conflicts of interest that had not been declared. Interests were assessed by a FIND panel including the TPP development group leadership team, and a FIND-DoI committee.

The decision to allow an external member to participate was made on the basis of whether any conflicts were specific, personal, and/or financially significant.

All members of the TPP development group were confirmed to not have any interests that could conflict with the objectives of the TPPs.

Two members were initially part of the development group but have not been listed due to their lack of participation in the TPP development process or failure to complete the required DoI forms.

List of abbreviations

Ct	Cycle threshold
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GTFCC	Global Task Force on Cholera Control
IFU	instructions for use
LMICs	low- and middle-income countries
NAAT	nucleic acid amplification test
PAMI	priority area for multisectoral intervention
PCR	polymerase chain reaction
RDT	rapid diagnostic test
TPP	target product profile
WHO	World Health Organization
WHO ERPD	WHO Expert Review Panel for Diagnostics

INTRODUCTION

Cholera is a severe diarrhoeal disease caused by the bacterium *Vibrio cholerae*. It is a major threat to global public health, with an estimated 1.3 to 4 million cases and 21 000 to 143 000 deaths occurring annually (1). The average cholera case fatality ratio reported globally in 2021 was 1.9% (2.9% in Africa), substantially above the accepted targeted rate (< 1%) and the highest recorded in more than a decade (2). Approximately 10% of individuals with cholera will develop severe symptoms, including acute watery diarrhoea and vomiting; without treatment, death can occur within hours (3).

There is thus a considerable burden of cholera cases globally. In addition, the world has been facing an acute upsurge in the seventh cholera pandemic since 2021; this is characterized by the number, size and concurrence of multiple outbreaks, the spread to areas free of cholera for decades and alarmingly high mortality rates (4). The simultaneous progression of multiple cholera outbreaks is compounded in countries that are experiencing complex humanitarian crises while relying on fragile health systems. This situation is further aggravated by climate change. The cholera pandemic thus presents a challenge to outbreak response, and there is the risk of the infection spreading further to other countries. Based on these factors, the increasing number of cholera outbreaks and their geographic expansion, in addition to the lack of vaccines and other relevant healthcare resources, the World Health Organization (WHO) assesses the current risk due to cholera globally to be very high (4).

Cholera disproportionally impacts the poorest and most vulnerable populations. Areas with poor sanitation, limited access to safe drinking water and deficient hygiene practices are at high risk for cholera transmission (3). In addition, limited access to healthcare facilities and inadequate treatment of cases are factors associated with a high level of cholera-related mortality.

The Global Task Force on Cholera Control (GTFCC) (5) and WHO continue to work with partners, including Gavi (6), at the global, regional and country levels. This work involves supporting member states in their efforts to control cholera. It comprises a long-term, multisectoral approach that integrates various interventions, including improved sanitation infrastructure, surveillance, and the introduction of oral cholera vaccine as an outbreak response and for preventive vaccination campaigns.

Recommendations for enhanced surveillance of cholera

Ending Cholera—A Global Roadmap to 2030 (5) (hereafter referred to as "the roadmap") was launched by GTFCC in October 2017. The roadmap targets a 90% reduction in cholera deaths overall and elimination of cholera transmission in 20 countries by 2030. In 2018, the roadmap was recognized by the 21st World Health Assembly and adopted by the WHO Regional Committee for Africa.

The strategy calls on countries and partners to focus on cholera "hotspots" or priority areas for multisectoral intervention (PAMIs). These are relatively small areas that are most heavily affected by cholera; they experience cases on an ongoing or seasonal basis and play a major role in the spread of cholera to other areas.

THE ROADMAP IS BASED ON THREE STRATEGIC AXES:

1. **EARLY DETECTION** and **QUICK RESPONSE** to rapidly contain outbreaks of cholera.
2. **IMPLEMENTATION OF A MULTISECTORAL APPROACH** targeted at high-risk areas. This includes the delivery of oral cholera vaccines; basic water, sanitation and hygiene (WASH) services; epidemiology and laboratory services; case management; and community engagement.
3. **EFFECTIVE COORDINATION** of technical support, **RESOURCE MOBILIZATION**, and **PARTNERSHIP** at local and global levels.

All three strategic axes of the roadmap are heavily dependent on the availability of reliable, accurate and high-quality epidemiological and laboratory surveillance data. Such data are necessary to detect, confirm and rapidly respond to cholera outbreaks; identify high-risk areas; monitor progress; assess (and adapt) prevention and control strategies; and substantiate the absence of cholera. The rapid identification of cholera PAMIs, based on existing surveillance data, is essential to tailor the correct combination of interventions that are best adapted to each context and population.

The objective of cholera surveillance and control strategies has long been limited to responding to and mitigating major epidemics. As a result, cholera surveillance has mainly relied on the reporting of clinically suspected cases and deaths, with no or limited laboratory confirmation. Therefore, the currently available cholera surveillance data are not sufficiently reliable to adequately support the objectives of the roadmap or to identify PAMIs across and within countries. Not all actual cases of cholera are reported; conversely, not all clinically suspected cholera cases are true cases, as the clinical signs of cholera are also commonly seen with other frequently occurring diarrhoeal diseases.

In 2024, GTFCC published updated recommendations and a series of supporting materials for strengthening public health surveillance for cholera, with the aim of having better informed, more timely and appropriately targeted multisectoral interventions (7, 8). The guidance states that laboratory testing should rely on testing strategies adapted to the prevailing cholera situation at the surveillance unit level (i.e. the presence or absence of a confirmed cholera outbreak) and to available resources; expanded use of rapid diagnostic tests (RDTs) to support the early detection of suspected outbreaks and incidence monitoring; and increased capacities for laboratory confirmation of cholera cases. Testing of suspected cholera cases should be routinely undertaken in accordance with systematic testing schemes, and again the use of RDTs should be expanded.

Current state of play of molecular diagnostic tools for cholera

Polymerase chain reaction (PCR) based on specific DNA sequences unique to a particular organism represents an alternative to culture and biochemical analyses for the identification of pathogens, including the *V. cholerae* strains responsible for cholera. The recently updated guidance from GTFCC (7) recommends that suspected cholera cases be confirmed either by culture or PCR. Molecular tests such as PCR are promising tools for cholera surveillance and outbreak confirmation, as they can have high sensitivity and specificity and rapid turnaround times. Molecular tests also enable the detection of epidemic-related features of a strain of *V. cholerae*, including the genes that encode cholera toxin (CT), an essential virulence factor of epidemic strains, and the two serogroups of *V. cholerae* associated with cholera (the O1 and O139 serogroups) (9).

Currently, however, many of the molecular assays in use are laboratory developed tests that are not standardized, making transfer of technology and comparisons of results between laboratories difficult. There is thus a need for commercialized molecular test kits for cholera designed for deployment and use in low- and middle-income countries (LMICs) at the level of regional/provincial or national laboratories to support cholera surveillance efforts in endemic countries and guide intervention strategies.

Existing molecular diagnostic tests for cholera have not been independently validated, and performance claims remain unverified. There are no molecular tests for cholera that have received WHO prequalification or been validated by a WHO expert review group. Some multiplex PCR tests that test for either *Vibrio* spp. or *V. cholerae* along with other pathogens have been authorized by the US Food and Drug Administration (FDA), although these tests are expensive and have not been independently validated.

To improve the quality and availability of laboratory data to ultimately reach the targets set out in the roadmap, there is an urgent need for easy-for-use, fit-for-purpose, well-performing and validated diagnostic tests.

The role of target product profiles

Target product profiles (TPPs) are strategic planning tools for guiding the development of new diagnostic tests and other healthcare products. The primary audiences for TPPs are manufacturers, suppliers and researchers developing new assays. A TPP outlines the key performance and operational characteristics that a product should possess to meet the needs of its intended users, target population and public health programmes, in its intended settings of use. For each characteristic, a TPP states a preferred criterion that is to be achieved by product developers if feasible and a minimal criterion if the preferred criterion is not feasible.

The first step towards accelerating the development of molecular diagnostics for cholera is to develop a TPP for molecular tests that are appropriate for cholera surveillance. Manufacturers of molecular diagnostics that meet the criteria detailed in the TPP will be eligible to submit an expression of interest to be evaluated by the WHO Expert Review Panel for Diagnostics (WHO ERPD).

The scope of the molecular TPP includes nucleic acid amplification test (NAAT) kits but does not cover instrument-and-cartridge diagnostic systems for fully automated testing. Although an assay for use on a fully automated diagnostic system could be useful if it satisfies the criteria defined in the TPP for molecular diagnostics for cholera set out below, the characteristics for such a system have been defined elsewhere and are not the subject of this document (see the TPP for a semi-open, multiplex, multi-analyte diagnostic platform (10)).

Molecular tests that are successfully assessed by WHO ERPD are expected to be prioritized for procurement to support enhanced cholera surveillance and inform a targeted oral cholera vaccine campaign strategy.

METHODS

This TPP was largely developed according to the standard WHO procedure (11), adapted as follows. Initial draft TPPs were developed by the TPP leadership team, considering similar TPPs, clinical and scientific literature and unmet clinical needs. Research, including a literature review, was conducted by the FIND staff listed in the Acknowledgements.

The authors established a TPP development group of 13 individuals, comprising scientists, experts, public health officials and representatives of intended users, who were selected according to the standard WHO procedure, with due attention paid to geographical and gender representation. The first meeting of the TPP development group involved discussions about the process and to establish the core characteristics of the TPPs.

In September 2023 the development group members received a draft of the TPP and were asked to complete a Delphi-like online survey to establish their level of agreement on each minimal and preferred characteristic criterion in the TPP. Their agreement rating was determined using a 4-point Likert-type scale: 1, fully disagree; 2, mostly disagree; 3, mostly agree; 4, fully agree; members could also mark “No opinion”. Comments were requested on all items and were required when members indicated that they did not agree (Likert score 1 or 2). Of 13 TPP development group members, 8 (62%) completed the survey. The levels of agreement (the count of responses of Likert score 3 or 4 divided by all Likert responses for a particular item), while not judged against a consensus threshold at this stage, were generally high, averaging 96% for minimal and 97% for preferred characteristics. All comments received were compiled and reviewed by the TPP leadership team, and the TPP was jointly revised to address criticisms, incorporate suggestions and avoid misunderstandings of intent. Subsequent meetings of the TPP development group were organized to review the development group survey results and agree upon the changes proposed to the TPP.

In January 2024 a public consultation was conducted. The same format as for the development group survey was used, with the additional requirement for consensus that ≥ 75% agreement of more than five non-abstaining responses was required for each minimal and preferred criterion. Any criterion that failed to reach consensus after two rounds of Delphi survey was, at the discretion of the development group, excluded or subject to caveats, with a summary of outstanding issues; the percentage agreement, including the number of respondents; and any recommendations for further research. In the first Delphi round, which lasted 44 days, a total of 23 responses were received (19 complete and 4 partial responses), with generally high agreement, averaging 92% and 94% for the minimal and preferred characteristics, respectively (Fig. 1 and Fig. 2). Consensus having been achieved, the TPP development group deemed a second Delphi survey unnecessary. The TPP development group reviewed the results of the survey, with minor clarifications and edits made, to produce the finalized TPP contained in this document.

Fig. 1. The 23 respondents to the public consultation, by WHO region and country

Figure 2. Level of agreement with the various TPP characteristics from the public consultation

SECTION	CHARACTERISTIC	AGREEMENT: MINIMAL	AGREEMENT: PREFERRED	RESPONSE COUNT
		100% 75% 50% 25% 0%	100% 75% 50% 25% 0%	
General	Goal of the test	87%	95%	22 1
	Target population	96%	91%	23
	Target use setting	87%	83%	23
	Test format	100%	94%	18 4
	Result display and interpretation	95%	95%	22
	Assay targets	94%	94%	17 2
User	Target users	84%	100%	19
	User training	89%	74%	19
Performance	Clinical sensitivity	89%	84%	19
	Clinical specificity	84%	89%	19
	Interference	100%	100%	19
	Specimen type and storage	88%	88%	17 2
	Specimen volume	82%	100%	16 3
Test procedure	Kit and reagent preparation	100%	100%	19
	Specimen preparation and extraction[1]	100%	93%	15 4
	Need for additional equipment	94%	94%	18 1
	Time to result	100%	100%	19
	Kit reagents	84%	100%	19
	Quality control	100%	100%	15 4
	Operating conditions	100%	94%	16 3
Operational	Test kit stability and storage conditions	89%	100%	19
	Shipping and transport conditions	88%	94%	17 2
	Open test kit stability	94%	94%	16 3
	Biosafety	88%	100%	16 3
	Waste disposal	89%	100%	18 1
Pricing and market access	Target list price per test[1]	87%	87%	15 4
	Quality management system	100%	100%	10 9
Average		92%	94%	18 1.7

FULLY AGREE
MOSTLY AGREE
AGREEMENT
MOSTLY DISAGREE
FULLY DISAGREE
GAVE OPINION
NO OPINION


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Target product profile for a molecular test for cholera surveillance

Please note that the numbering of notes restarts after each row.

CHARACTERISTIC	MINIMAL	PREFERRED
Goal of the test	A surveillance test[1] to confirm and monitor outbreaks of cholera and confirm the end of outbreaks of cholera (e.g. *V. cholerae* 01 and 0139 or toxigenic *V. cholerae* 01 and 0139, according to the epidemiological context).[2,3]	Same as minimal, plus further characterization and identification of a cholera pandemic strain.
	[1] According to WHO definitions of test purposes (11). The tests described under both the minimal and preferred criteria are for surveillance purposes; they are not meant to inform patient management decisions, only to identify cholera cases. [2] Testing outputs will also inform estimates of disease burden and reactive vaccination campaigns. [3] *V. cholerae* 0139 is uncommon, therefore confirmatory testing remains important as RDTs may provide *V. cholerae* 0139-positive results that require confirmation.	
Target population	All patients who meet the clinical definition of a suspected case of cholera (7).	Same as minimal plus cases not meeting the clinical case definition but that are part of an ongoing epidemiological investigation or are linked to a previously confirmed case of cholera.
Target use setting	Specimens collected in primary healthcare settings, including health posts (healthcare level 1 and above).[1] Test performed in healthcare level 4 settings with existing molecular laboratory capacity.	Specimen: same as minimal Test performed in healthcare level 3 and 4 settings with existing molecular laboratory capacity.
	[1] For definitions of healthcare levels, see Table 1.	
Test format	Nucleic acid amplification test (NAAT) kit validated on commercially available thermocyclers.	
Result format and interpretation	Qualitative result (e.g. detected or not detected). The instructions for use (IFU) must state the process required for interpretation, such as the signal exceeding a Ct value as defined by the manufacturer.	
Assay targets	One target conserved across circulating *V. cholerae* 01 strains and one target conserved across 0139 strains (e.g. *rfb01* and *rfb0139* are currently recommended), plus a third target appropriate for confirming the presence of the cholera toxin gene (*ctxA* is currently recommended). Assay gene targets must be specified in the IFU.[1,2]	Same as minimal, plus the following, in order of most to least preferred: - identify the species *V. cholerae* (e.g. *ompW, toxR*, ISR) - identify and distinguish biotype (El Tor or classical) - identify whether it belongs to the seventh pandemic lineage
	[1] Cholera toxin testing recommendations are detailed in the GTFCC surveillance guidelines. Unlike *ctxB*, *ctxA* is highly conserved, thus it is the recommended marker for the toxin gene. Detection of the toxin gene alone is not sufficient to identify cholera (7, 12, 13). [2] Preferably, assay targets should be combined in a single test as follows, in order of most to least preferred: 01 + 0139 + cholera toxin; 01 + cholera toxin; or 01 + 0139. (0139 only or 0139 + cholera toxin are not appropriate for current surveillance purposes). Although cholera toxin only tests are not routinely used on their own, according to the GTFCC surveillance guidelines (7), they may be used to confirm outbreaks in specific circumstances.	

CHARACTERISTIC	MINIMAL	PREFERRED
USER REQUIREMENTS		
Target users	Unprocessed stool specimen[1] collected by laboratory personnel or non-laboratory trained healthcare providers. Test performed by trained laboratory personnel.	Same as minimal, plus: Specimen can be collected by a community health worker. Test can be performed by any trained staff member in a healthcare facility.
	[1] Unprocessed stool specimens will need to be transported from healthcare level 1 to level 3 or 4 for processing and testing (see specimen storage, below). For definitions of healthcare levels, see Table 1.	
PERFORMANCE CHARACTERISTICS		
Clinical sensitivity	≥ 95% per assay target	≥ 97% per assay target
	A molecular reference standard has not been established, so the manufacturer should develop their approach carefully and explain their rationale in the IFU.	
Clinical specificity	≥ 97% per assay target	≥ 99% per assay target
	See the note for clinical sensitivity.	
Interference	No interference from *V. cholerae* non-O1/non-O139 or other pathogens that cause common human diseases, especially those presenting with similar signs and symptoms, e.g. watery diarrhoea, or from common endogenous or exogenous interferents.[1]	
	[1] Interferents should be tested at clinically relevant concentrations, included in a risk evaluation and listed in the IFU. See CLSI EP07 (14). See Table 2 for a list of relevant interferents to be considered.	
TEST PROCEDURE CHARACTERISTICS		
Specimen type[1]	The test must be validated for at least one of the following specimen types: Unprocessed stool Rectal or faecal swab in Cary–Blair medium	All specimens listed in minimal, plus any of the additional specimen types: Isolates from stool culture Stool on wet filter paper Stool on dry filter paper Stool in alkaline peptone water
	[1] Specimen storage is described in the GTFCC job aid, which for all specimen types recommends storage at ambient temperature (see operating conditions, below) and ideally at 22–25 °C (15).	
Specimen volume	< 1 mL	
Kit and reagent preparation	Benchtop preparation, including reagent reconstitution and transfer of specimen.	Same as minimal, but all reagents are ready to use and require no additional preparation by the user.
Specimen preparation and extraction[1]	Nucleic acid from specimen obtained through either boiling lysates or a manual extraction process. All necessary supplies for extraction provided in the kit or commercially available. Extracted material storage conditions and duration must be defined in the IFU.[1]	Same as minimal, but if a manual extraction process is required, a simple extraction process is preferred with minimal steps that does not require centrifugation by the user, e.g. swab elution in lysis buffer.
	[1] If commercially available extraction methods are part of the test procedure, they must be validated by the manufacturer for this purpose and listed in the IFU.	
Need for additional equipment	If nucleic acid extraction is required, the method must be compatible with widely used manual and automated workflows, with no need for additional proprietary extraction instrumentation. Assay compatible with off-the-shelf equipment for amplification and detection, i.e. at least one widely used thermocycler.	Same as minimal, plus assay compatible with and validated on multiple widely used thermocyclers.

CHARACTERISTIC	MINIMAL	PREFERRED
Time to result	≤ 4 h including assay run-time but excluding pre-analytical and extraction steps.	Same as minimal, except ≤ 2 h
Kit reagents	Frozen reagents in separate vials for each kit component are acceptable.	All reagents must support transport without cold-chain. All master-mix reagent components preassembled for each reaction.
Quality controls[1]	Internal controls for reaction inhibition and nucleic acid extraction (endogenous or exogenous); positive and negative controls are provided in the kit or are sold separately (directly by the NAAT kit supplier).	Internal controls for specimen adequacy, reaction inhibition and nucleic acid extraction are all included.

[1] The range of acceptable internal control results should be stated in the IFU.

OPERATIONAL CHARACTERISTICS		
Operating conditions	10–35 °C and ≤ 90% non-condensing humidity at elevations ≤ 2500 m.	10–40 °C and ≤ 95% non-condensing humidity at elevations ≤ 4000 m.
Test kit stability and storage conditions	12 months at either 4–35 °C or ≤ -15 °C, by choice of the manufacturer, and ≤ 70% non-condensing humidity at elevations ≤ 2500 m.	24 months at 2–40 °C and ≤ 90% non-condensing humidity at elevations ≤ 3000 m. Indicators of temperature and humidity excursions that would result in invalid or low-performance results included.
Shipping and transport conditions	72 h at either 2–8 °C or ≤ -15 °C, by choice of the manufacturer, with ≤ 1 h excursions to 37 °C.	72 h at 2–50 °C fluctuating.
Open test kit stability	Once reagents are thawed or opened, ≥ 24 h at 2–8 °C. If reagents are loaded onto an analyser upon opening, ≥ 7 days.	Once reagents are thawed or opened, ≥ 90 days at 2–8 °C or ideally at 2–40 °C. If reagents are loaded onto an analyser upon opening, ≥ 90 days.
Biosafety	Standard safety precautions for specimen collection recommended. All materials are free of substances with a Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classification of H340, H350 or H360, with minimal inclusion of any materials with other GHS "H" classifications (16). The test can be performed under core biosafety requirements, similar to those previously referred to as biosafety level 2, with heightened control measures applied based on local risk assessment.	Same as minimal, but with reduced biosafety requirements, e.g. specimen collection/preparation includes buffer-based (non-heat) lysis and inactivation, or specimen enters a closed system.
Waste disposal	Standard biohazard waste disposal or incineration of consumables.	All components of the kit are designed to minimize the environmental impact during standard biohazard waste disposal.

See (17)

PRICING AND MARKET ACCESS		
Target list price per test[1]	≤ US$ 11 per reaction per target[2]	≤ US$ 8 per reaction per target[2]

[1] Pricing from manufacturers should be as low as sustainably possible while maintaining quality, based on evidence of the true cost of goods sold accounting for material, manufacturing process, operational logistics and commercialization efforts. Pricing should also include and clearly define all facets of end-to-end implementation (e.g. support, maintenance). Pricing must account for production at scale with defined volume thresholds. Ultimately pricing should intersect sustainable long-term viability for the manufacturer with affordability to support widespread access to testing in LMICs and should be transparently published.
[2] This price includes the cost of extraction and excludes any costs for instruments.

Quality management system	Compliant with ISO 13485	Certified to ISO 13485 or equivalent

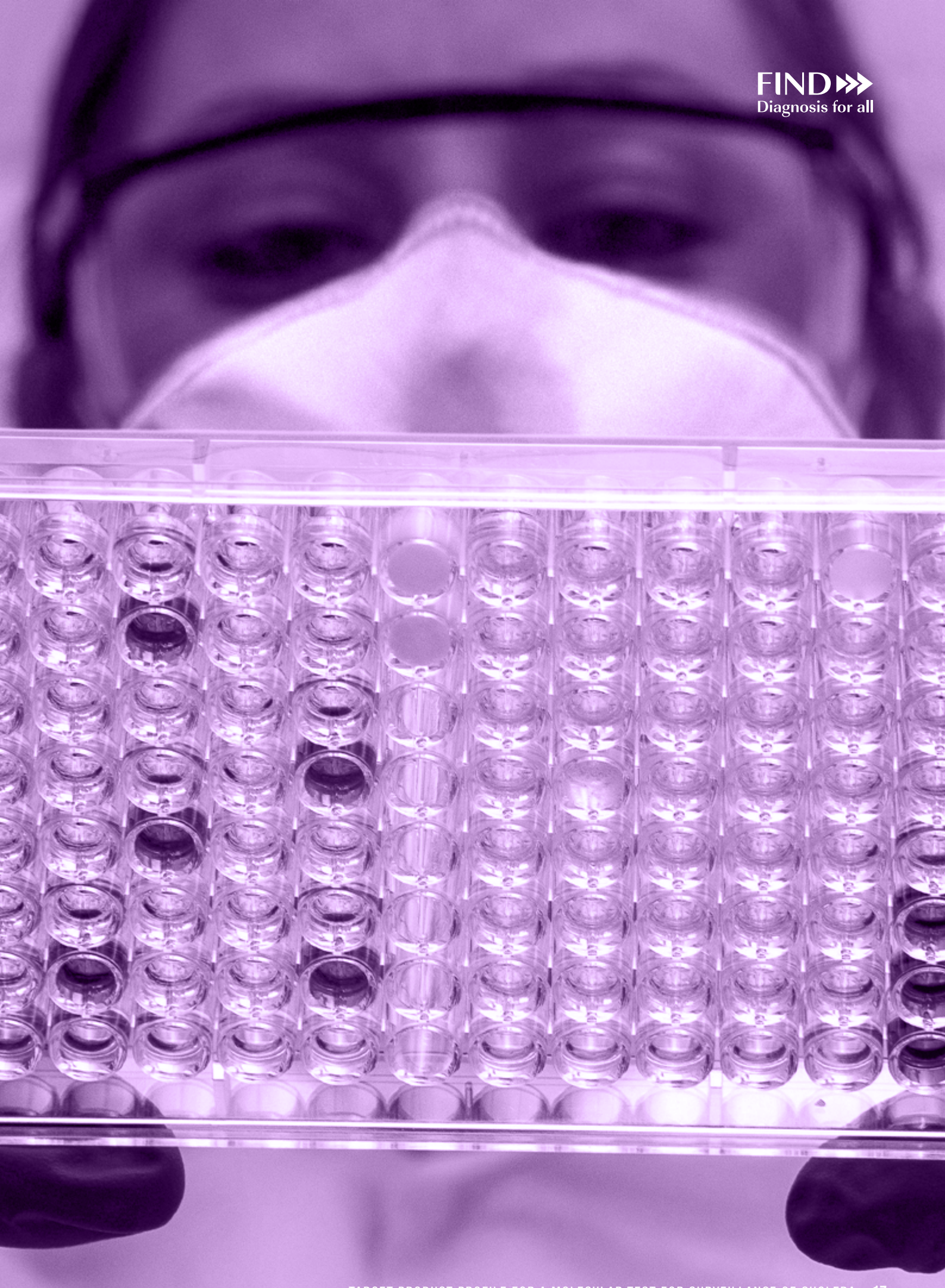
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Table 1. Definitions of use settings in LMICs (adapted from Ghani et al. (2015) (18))

	SELF-TESTING	LEVEL 0 (L0) - COMMUNITY	LEVEL 1 (L1) - PRIMARY CARE
Use setting	• Self-testing	• Community outreach • Home testing	• Primary care facility
Laboratory infrastructure	• No mains power • No water • No laboratory equipment • No environmental control, (e.g. temperature, dust, humidity)	• No mains power • No water • No laboratory equipment • No environmental control, (e.g. temperature, dust, humidity)	• No mains power (unreliable) • Minimal laboratory equipment (may not support cold chain) • BSL-1 containment • No environmental control (e.g. temperature, dust, humidity)
Operator skill	• Self-testing ▶ Simple reagent/sample transfer	• Nurse/pharmacist • Community health worker ▶ Simple reagent/sample transfer	• Nurse • Trained laboratory worker ▶ Minimal specimen processing (≤ 3 steps)
Specimen capacity	• Can process minimally invasive samples: fingerstick blood, nasal swabs, saliva, urine	• Can process minimally invasive samples: fingerstick blood, nasal swabs, saliva, urine	• Can process upper respiratory specimens; may not have capacity for lower respiratory, venipuncture, plasma
Test capacity	• True POC MDx (some) • RDT	• True POC MDx (some) • RDT	• True POC MDx • Basic microscopy • RDT

BSL, biosafety level; CLIA, chemiluminescent assay; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; LDT, laboratory developed test; MDx, molecular diagnostic test; POC, point of care; PRNT, plaque reduction neutralization test; RDT, rapid diagnostic test.

LEVEL 2 (L2) - DISTRICT HOSPITAL LABORATORY	LEVEL 3 (L3) - REGIONAL/PROVINCIAL LABORATORY	LEVEL 4 (L4) - REFERENCE/NATIONAL LABORATORY
• Near-patient laboratory • Referral hospital laboratory • Emergency department testing	• Near-patient laboratory • Referral hospital laboratory • Emergency Department testing	• Reference laboratory
• Mains power (may be intermittent) • Basic laboratory equipment (biosafety cabinet, centrifuge, calibrated pipettes, refrigerator) • –20 °C freezers (some) • BSL-1/2 containment (some) • Environmental control (e.g. temperature, dust, humidity) (some)	• Mains power (may be intermittent) • Basic laboratory equipment (biosafety cabinet, centrifuge, calibrated pipettes, refrigerator) • –20 °C freezers • BSL-1/2 containment • Environmental control (e.g. temperature, dust, humidity)	• Mains power (reliable) • High infrastructure facility • –20 °C freezers • –80 °C freezers (some) • BSL-2/3 containment • Environmental control (e.g. temperature, dust, humidity)
• Laboratory technician (certified for 1–2 years) ▸ Sample processing with calibrated volumes (≤ 3 steps)	• Laboratory technician (certified for 1–2 years) ▸ Sample processing with calibrated volumes (≤ 3 steps)	• Scientific research specialist • Laboratory technician (certified for 1–2 years)
• Can process most BSL-2 specimens; depends on clinic's sample capacity	• Can process most BSL-2 specimens; depends on clinic's sample capacity	• Can process most BSL-2/3 specimens
• Near-POC MDx • ELISA with a simple reader (some) • Microscopy • RDT • Clinical chemistry (some)	• Blood culture and microbiology capacity (some) • Near-POC MDx • ELISA with a simple reader • Microscopy • RDT • Clinical chemistry	• Blood culture and microbiology capacity • Lab MDx/PCR/LDT • ELISA/EIA/CLIA/PRNT • Fluorescence microscopy • Clinical chemistry • Sequencing (some) • Mass spectrometry (some)

Table 1. List of potential interferents, as applicable

POTENTIAL CROSS-REACTIVE PATHOGENS	OTHER POTENTIAL INTERFERENTS
Vibrio cholerae non-O1/non-O139 and non-pandemic clones	Human whole blood, bile or urine
Vibrio parahaemolyticus	Triglycerides
Vibrio alginolyticus	Cholesterol
Vibrio mimicus	Fatty acids
Vibrio fluvialis	Bovine mucin
Plesiomonas shigelloides	Human stool (overfill of Cary–Blair vial)
Escherichia coli (including types responsible for diarrhoeal diseases)	Anti-diarrhoeal/laxative medications
Yersinia enterolitica	Tetracyclines, fluoroquinolones and macrolides used to treat cholera infection or other antibiotic
Shigella sonnei	Antimalarial, antiretroviral and antituberculosis medications
Shigella flexneri	Common over-the-counter anti-inflammatory and analgesic medications such as paracetamol and ibuprofen
Salmonella typhi	Culture media used for downstream laboratory analysis
Salmonella enteritidis	Decontamination products used in specimen collection (bleach, ethanol, etc.)
Salmonella typhimurium	
Aeromonas hydrophila	
Aeromonas sobria	
Campylobacter coli	
Campylobacter jejuni	
Clostridium difficile	
Rotavirus, enteric adenovirus, norovirus, astrovirus and other viruses responsible for diarrhoeal diseases	
Additional *Enterobacteriaceae* responsible for diarrhoeal diseases	

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