GLOBAL TASK FORCE ON CHOLERA CONTROL

# Interim Technical Note
# The Use of Cholera Rapid Diagnostic Tests
# November 2016

## Objective

To provide interim guidance to Ministries of Health and other organizations on the potential use of commercially available rapid diagnostic tests for detection and surveillance of cholera.

## Background

The usual approach for patient diagnosis and for surveillance of cholera is based on clinical examination of suspected cholera cases, with confirmation by positive culture of stool specimens in reference laboratories. Culture is very specific and is considered the reference-standard method in most countries[1]. In addition, isolation of *Vibrio cholerae* (VC) strains has the advantage of allowing further phenotypic analysis of the isolates for antibiotic susceptibility testing, and genetic as well as further molecular characterization (by molecular subtyping techniques). However, poor quality of sampling and delays in shipment could significantly affect the efficacy of culture as a primary diagnostic tool[1]. Molecular testing has become an alternative to culture for identification of many pathogens and PCR methods have been developed for the identification of *V. cholerae* species and characterization of O1 and/or O139 serogroups.

From a public health perspective, the management of cholera outbreaks requires immediate identification due to the pathogen's potential for spread and devastating consequences of epidemics. However, in many endemic countries the ability to use culture - or PCR-based diagnostic approaches is restricted by insufficient, or lack of appropriate, laboratory capacity. This is due to problems in staff management, lack of trained laboratory staff, and unavailability of laboratory supplies, challenges with storage and transport of samples, unreliable reporting, and overall financial constraints. In particular, culture confirmation is rarely accessible at peripheral health care facilities where most cholera patients present. These challenges result in delays in outbreak detection and subsequent control measures, and ultimately lead to unreliable estimates of the cholera burden worldwide [2].

Rapid Diagnostic Tests (RDTs) for the detection of *V. cholerae* O1 and/or O139, the causative agents of cholera, have been marketed as an alternative to culture or PCR for the confirmation of clinically suspected cholera cases in situations where access to appropriate laboratory services is not readily available. There are more than 20 cholera RDTs currently marketed, mainly based on detection of O1 and O139 antigens in human stool specimens using monoclonal antibodies [3,6,7,8,9]. Cholera RDTs are an immunochromatographic test device – a dipstick that is dipped into a tube containing the

specimen, or a cassette where the specimen is applied to a sample well. They can be used by trained non-laboratory personnel at the patient's bedside in peripheral settings, outside of laboratory premises[5]. They provide a rapid (within a maximum of 30 minutes) qualitative result (colored band) that can be read with the naked eye.

Cholera RDTs are used to quickly identify cholera cases in a population, while efforts to confirm the outbreak by culture and PCR continue. The use of cholera RDTs could potentially augment the capacities of countries to more rapidly detect suspected cholera cases and thereby improve cholera surveillance[3,4,5].

This interim technical note is intended to provide Ministries of Health and other organizations with technical information on cholera immunologically-based RDTs, and reviews indications for their practical use in the field.

## Performance and limitations of Cholera RDTs

Cholera RDTs belong to a category of in vitro diagnostic products which are classified as lower risk for regulatory oversight. Therefore, the stringency of independent reviews of product is less than for higher risk products, even within the same regulatory jurisdiction. As a result, the quality of the products is not standardized. In addition, there are issues reported of products being continuously modified, often quite substantially, without prior information, leaving end-users unaware of required adjustments.

Furthermore, only a few products have been evaluated by academic and research institutions independently of the manufacturers.

Evaluations conducted on the most recent versions of commercially available cholera RDTs, highlighted large variations of their performance with regard to:

- sensitivity, ranging from 58 to 100%
- and specificity, reported to range between 60 and 100%[7] depending on the test and the setting, even when taking into account the limitations in the study methodology that might have led to underestimate the specificity[7,10].

## When to use Cholera Rapid Diagnostic Tests

- RDTs are intended to be used at primary health care level for surveillance purposes, in peripheral health care facilities. RDTs increase the specificity of clinical diagnosis of cholera and improve its positive predictive value by permitting the triage of specimens for laboratory confirmation.
- Cholera RDTs may be used for:
  - early outbreak detection, as a tool for an initial alert,
  - monitoring of outbreaks,
  - monitoring of seasonal peaks in highly endemic areas.
- In all situations, cholera RDTs should be performed on clinically suspect cholera cases only.

- In areas where confirmed cholera cases have not been recently reported, if one or more patient(s) clinically suspect of cholera return a positive RDT result, this is sufficient to immediately launch a cholera alert, send stool specimen to the reference laboratory for confirmation, and initiate response measures (e.g. inform authorities, mobilize resources and material, etc.)[1].
- In areas with ongoing outbreaks, positive RDTs can be used to select stool specimens from suspected cases for culture.
- RDTs are not a substitute for stool culture: any positive RDT(s) result must be confirmed by culture or PCR as soon as possible before confirming the alert and declaring a cholera outbreak. Culture or PCR/molecular testing allow identification, but also characterization and genotyping of the circulating strains, which is part of the surveillance of cholera disease and is useful for epidemiological purposes.
- If all RDTs are negative, cholera should be ruled out.
- Cholera RDTs are of limited usefulness for individual diagnosis among suspected cholera patients, since the results of the test would have no influence on the immediate management of the case.

## Notes for cholera RDT implementation

- A test kit usually includes the individually packed tests, the corresponding material needed to perform the tests and a technical leaflet with the instructions for use (in English, French and Spanish), which are specific to each brand.
- The instructions for use provided with the test kit by the manufacturer should be used to guide the end-users on how to organize the long-term storage of cholera RDTs. Most of the commercially available devices do not need a cold-chain for storage or transport. Nevertheless, the stability of the tests is only guaranteed up to a maximum of 35°C – 40°C and the shelf-life varies from 12 to 18 months, depending on the manufacturer and the product.
- When performing the test, the manufacturer's instructions should be strictly followed, in particular the guidance on how to collect and store specimens.
- At the time of introduction in each country, the tests should ideally be evaluated at the central level in the National Reference Laboratory on a set of culture or PCR-confirmed positive and negative patient specimens if available, before their distribution and use. It is recommended that use of RDT be described in country specific Standard Operating Procedures developed by the regional and/or national reference laboratory and taking into account language, country regulations and quality management system, etc.
- Although cholera RDTs are easy to use, training sessions should be organized at the locations where the tests will be introduced so that laboratory staff as well as non-laboratory staff understand the required methods for specimen collection, test kit

[1] It is worthwhile mentioning that visualization of the typical darting motility of *V. cholerae* by direct microscopic examination of fresh stool may be useful as complementary screening methods to RDT results.

storage, how to perform the test procedure and interpretation and management of test results. It is especially important not to exceed the recommended reading time (usually 15-20 minutes) as this may give false positive results. When interpreting the test result, the positivity of the control line must be checked systematically (colored signal). The absence of signal in the control line invalidates the test, which should therefore be repeated.

- The best results are obtained from fresh liquid stools or rectal swabs. The decision on which specimen to be collected should be guided by the manufacturer's instructions for use. Specimens should be collected and stored in clean containers without disinfectants (bedpans are not a suitable collection container). False negative results can occur (and sensitivity be reduced) if the specimens are collected i) in receptacles containing chlorine residues, ii) after initiating antibiotic therapy or iii) in case of poor sampling or handling practices of the specimen (e.g. long delay)[11].
- Stool specimens referred for culture and/or PCR should be stored in transport medium such as Cary Blair or filter paper and sterile saline[12] and kept at ambient temperature. Likewise, it is not recommended to refrigerate stool specimen when sending to the reference laboratory, as cold storage conditions (less than 5°C) can greatly decrease the populations of VC.

## Selection of Cholera RDTs

Many cholera RDTs are available from various companies worldwide. Interested end-users should check the following test specifications to select the test that is most appropriate for their setting and diagnostic requirements:

**Detection target:** the capacity to distinguish between VC O1 and VC O139 antigen may be of interest in areas where the two serogroups are/have been reported. In other areas a test for detection of VC O1 only is sufficient.

**Review of performance**: the review of independently conducted performance evaluations is the best approach to select a well-performing product and users should refer to recent evaluations for their choice (see Evaluations of Cholera Rapid Diagnostic Tests references [13-22]). It is better to use RDTs whose performance has been evaluated in the setting of intended use, i.e. field- and population-based evaluations. Performance testing conducted on isolated VC strains rather than faecal specimens should be interpreted with caution as this approach may lead to an overestimation of test performance.

**Expected minimal performance**: cholera RDTs should have a sensitivity of at least 90% and a specificity of at least 85% limiting the proportion of false positives to an acceptable level.

**Manufacturing of cholera RDTs**: The Global Task Force for Cholera Control urges manufacturers to continue to develop tests that meet high standards for production with

relevant quality management system including post-market surveillance measures (lot testing, complaint reporting, etc.) and highlights the importance to engage in a pre-qualification process. Interested partners and countries are invited to contact WHO and the Global Task Force for Cholera Control for further information or advice and support (GTFCCsecretariat@who.int).

## References

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**Evaluations Cholera Rapid Diagnostic Tests**

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15. Performance and utility of a rapid diagnostic test for cholera: notes from Haiti (Diagn Microbiol Infect Dis, 2013)
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18. Evaluation of a Rapid Test for the Diagnosis of Cholera in the Absence of Gold Standard (PLoS ONE 2012)
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