Nepal Government
Ministry of Health and Population
Department of Health Services
Epidemiology and Disease Control Division

RDT based Cholera Surveillance
Standard Operating Procedure 2024

1. Background:

Cholera is an acute diarrheal infection caused by ingestion of food or water contaminated with the bacterium *Vibrio cholerae*. Out of various serogroups of cholera only two V. cholerae—O1 and O139—are known to cause epidemics. The spread of cholera is strongly associated with insufficient access to sanitary facilities and clean water.

Cholera can be endemic or epidemic. A location where confirmed cases of cholera with evidence of local transmission have been found within the last three years (i.e., cases are not imported from elsewhere) is considered cholera-endemic. Endemic nations and those where cholera is not frequent can experience a cholera outbreak or epidemic.

The global burden of cholera in endemic nations showed that the incidence rate of cholera is 1.64 per 1000, which is more than 60% of the Nepalese population at risk of contracting the disease i.e. over 18 million people. (Rhee, et al., March 2020.)

Cholera rapid diagnostic tests (RDT)

Cholera rapid diagnostic tests (RDT) are a crucial tool for early detection of cholera cases. Adapted use of RDTs offers an enhanced avenue for early outbreak surveillance. RDTs were first used in Nepal as research cum pilot in EWARS and non-EWARS selected sites with the support from Johns Hopkins University (JHU) and International Vaccine Institute (IVI), Group for Technical Assistance (GTA).

As per the GTFCC, RDTs are intended to be used for surveillance purposes, not for case management. They are a tool for triaging samples to be further tested in laboratories for outbreak detection and to help monitor incidence trends of true cholera in surveillance units when there is a confirmed cholera outbreak.

Epidemiology and Disease Control Division (EDCD), Department of Health Services (DOHS) has decided to continue using RDT in cholera surveillance. RDTs used in Nepal are supplied through Gavi and its use in cholera surveillance is recommended by GTFCC, however, this is yet to be pre-qualified by WHO.

Information on Cholera RDT that will be used in Nepal.

Name: *Crystal VC-Immunochromatographic One Step Rapid Visual Test for Vibrio cholerae dipstick (O1 and O139)*

Production: Arkray Healthcare Pvt. Ltd.

Director General

Shelf life: 16 months

Reagent storage: Store the kit in cool and dry place and protect it from direct sun light. The kits should be stored between +4 ºC to +30 ºC. Do not Freeze.

Sensitivity and Specificity

The overall sensitivity and specificity of the RDT kits used in Nepal for detection of O1 and O139 in human stool was estimated by comparing its results with culture/PCR technique for the detection of *V. cholera* O1 and O139.

% Sensitivity	95 % CI
98.21	94.87 - 99.63

% Specificity	95 % CI
93.08	91.41 - 94.51

Source: Arkray Healthcare Pvt.Ltd, Crystal VC-Immunochromatographic One Step Rapid Visual Test for Vibrio cholerae dipstick (O1 and O139)

In areas where cholera prevalence is less than 5% when there is not an ongoing outbreak, like Nepal, the negative predictive value (NPV) is extremely high, over 99%. This means that when cholera RDTs produce a negative result in this context, there is less than a 1% chance that the test is incorrect. Cholera RDTs therefore are an effective method for reducing diagnostic costs spent on patients without cholera.

While the probability of having a false negative RDT is extremely low, the chances of having a *false positive test* become a slightly greater concern in these low prevalence settings as positive predictive value (PPV) decreases.

For this reason, the program will not *declare an outbreak based on a direct RDT alone in this context*. To mitigate the chances of having a false positive, specimens that produce direct positive RDTs should be confirmed with stool culture or PCR in the respective laboratories.

2. Case definitions for Surveillance:

Suspected Cholera Case/AWD Cases:

A suspected cholera case is a person aged two years or older having three or more loose stool within 24 hours in past 7 days with any form of dehydration and an exclusion of blood in stool; or who died from acute watery diarrhea with no other known cause of death.

Note: This definition is adopted in Nepal with a reference to the definition recommended by GTFCC.

Probable Cholera:

A probable cholera case is defined as a suspected cholera case with a positive rapid diagnostic test (RDT+).

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Confirmed Cholera:

A confirmed cholera case is any person infected with Vibrio cholerae O1 or O139, as confirmed by culture (including seroagglutination) or PCR.

3. Testing Procedure:

Selection of Patient:

All the suspected cholera/AWD case that meets the following criteria shall be screened at Emergency, OPD, and inpatient.

Inclusion Criteria:

1. Age ≥2 years
2. ≥3 loose stools in <24 hours
3. Duration of illness less than 7 days
4. Any form of dehydration in OPD or Inpatient or
5. Patient present with 1 to 3 in Emergency Department

To assess the dehydration status please refer to WHO Dehydration Assessment Chart as below;

Clinical assessment for degree of dehydration associated with diarrhea is as follow

- If two or more of the signs in column C are present – the patient has severe dehydration

Categories	A	B	C
General appearance	well, alert	restless, irritable	lethargic or unconscious
Eyes	normal	sunken	sunken
Thirst	drinks normally, not thirsty	thirsty, drinks eagerly	drinks poorly, or not able to drink
Skin turgor	goes back quickly	goes back slowly	goes back very slowly

- If two or more of the signs in column B (and C) are present – the patient has some dehydration
- Patients who fall under column A – "no signs of Dehydration

Exclusion Criteria:

1. Blood in stool

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Procedure for Collection of Sample:

Fecal specimens such as liquid stool should be collected in a clean container that is free of disinfectant or detergent residue. Specimens should not be collected from bedpans as these may contain residual disinfectant or other contaminants.

If a stool specimen cannot be produced, rectal swabs may be collected and enriched in Alkaline Peptone Water (APW) for 4-6 hours at 37 °C before RDT testing. To the extent possible, fecal specimens should be collected in the early stage of the illness when pathogens are usually present in the stool in highest numbers (i.e., within the first four days of illness, and before antibiotic therapy has been initiated).

Rehydration treatment of patients should not be delayed for specimen collection. Specimens may be collected after rehydration protocols have been initiated.

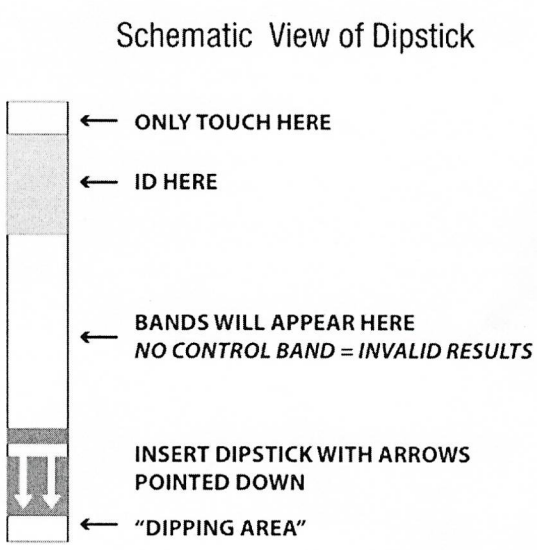
Procedure for Performing a cholera RDT:

Before you start

- Check the expiry date. If expiry date has passed, use another kit.
- Read carefully the instructions for use in its entirety.
- Ensure the reagent bottle is intact and solution is not turbid or discolored. Discard bottle if unsatisfactory.

Procedure:

1. Wear appropriate personal protective equipment.
    - Put on the gloves. Use new gloves for each patient.
2. Open the cap of the sample processing vial or specimen collection tube. Label tube with patient identifier or patient ID.
3. **Solid fecal specimens**: Collect the sufficient fecal specimens using the specimen collection swab.



Liquid fecal specimens: Draw liquid fecal specimens up to the fill line using disposable dropper.

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

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5. Break or open the outer end of the cap (point away or cover with tissue to avoid splash). Dispense 4 drops of processed sample into labelled 5 ml test tube.
6. Carefully open test pouch. Discard if damaged, or if desiccant is missing or changed in color. Write patient's name on the top of the dipstick.
7. Place the dipstick in the test tube with the arrows facing down. Confirm the end of the dipstick is submerged in the processed sample.
8. Wait for 15-30 minutes.

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RAPID DIAGNOSTIC TEST (RDT) FOR CHOLERA DETECTION

Quick Reference Guide - *For more detailed instructions please refer to the manufacturer's Package Insert*

GLOBAL TASK FORCE ON CHOLERA CONTROL

Indication of use

- RDTs are not used for individual diagnosis.
- RDTs are used as a tool **for early outbreak detection only** and once the outbreak is declared **for triaging the samples** to be sent to the laboratory.
- Perform RDT on fresh stool specimens and process within 2 hours collection.

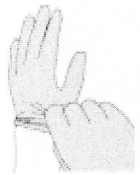
Before you start

- Check the expiry date. If expiry date has passed, use another kit.
- Read carefully the instructions for use in its entirety.
- Ensure the reagent bottle is intact and solution is not turbid or discoloured. Discard bottle if unsatisfactory.

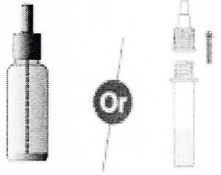
At the end

- Place all waste in a double-lined plastic bag labelled "Biohazard."
- Record the test results in the patient's information record or registers.
- Keep samples under adequate conditions and send them to the laboratory for culture or PCR (see GTFCC packaging and shipping lab aids).
- Report result accordingly.

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



2 Open the cap of the sample processing vial or specimen collection tube. Label tube with patient identifier.

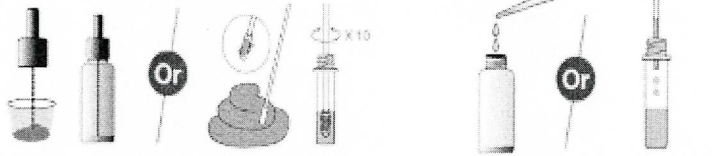


Sample processing vial

Specimen collection tube

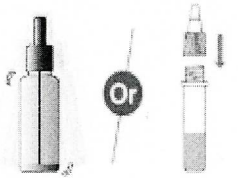
3 **Solid fecal specimens:** Collect the sufficient fecal specimens using the specimen collection swab.

Liquid fecal specimens: Draw liquid fecal specimens up to the fill line using disposable dropper

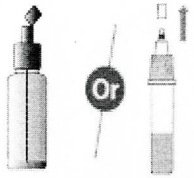


Discard the swab or dropper in the sharps container or double-lined plastic bag labelled "biohazard" after adding specimen

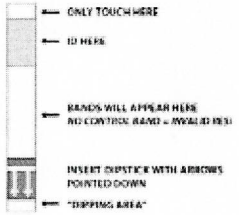
4 Tightly recap sample processing vial or collection tube and shake to mix contents



5 Break or open the outer end of the cap (point away or cover with tissue to avoid splash). Dispense 4 drops of processed sample into labelled 5 ml test tube



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

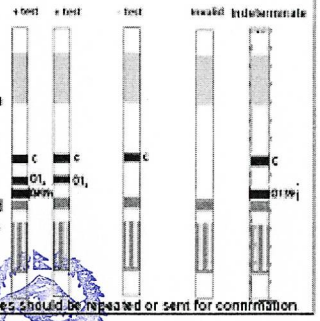


Dipstick

7 **Dipstick:** Place the dipstick in the test tube with the arrows facing down. Confirm the end of the dipstick is submerged in the processed sample

Test tube with dipstick

8 **Dipstick: Wait 15-30 minutes.**
Remove dipstick and read the result



As each RDT type, even from the same manufacturer, may have different positions for positive and control lines on the strip, please use the instructions provided with the specific RDT in use for correct interpretation. Example→

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

Tests that only have control and O139 lines should be repeated or sent for confirmation

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4. RDT testing Algorithm

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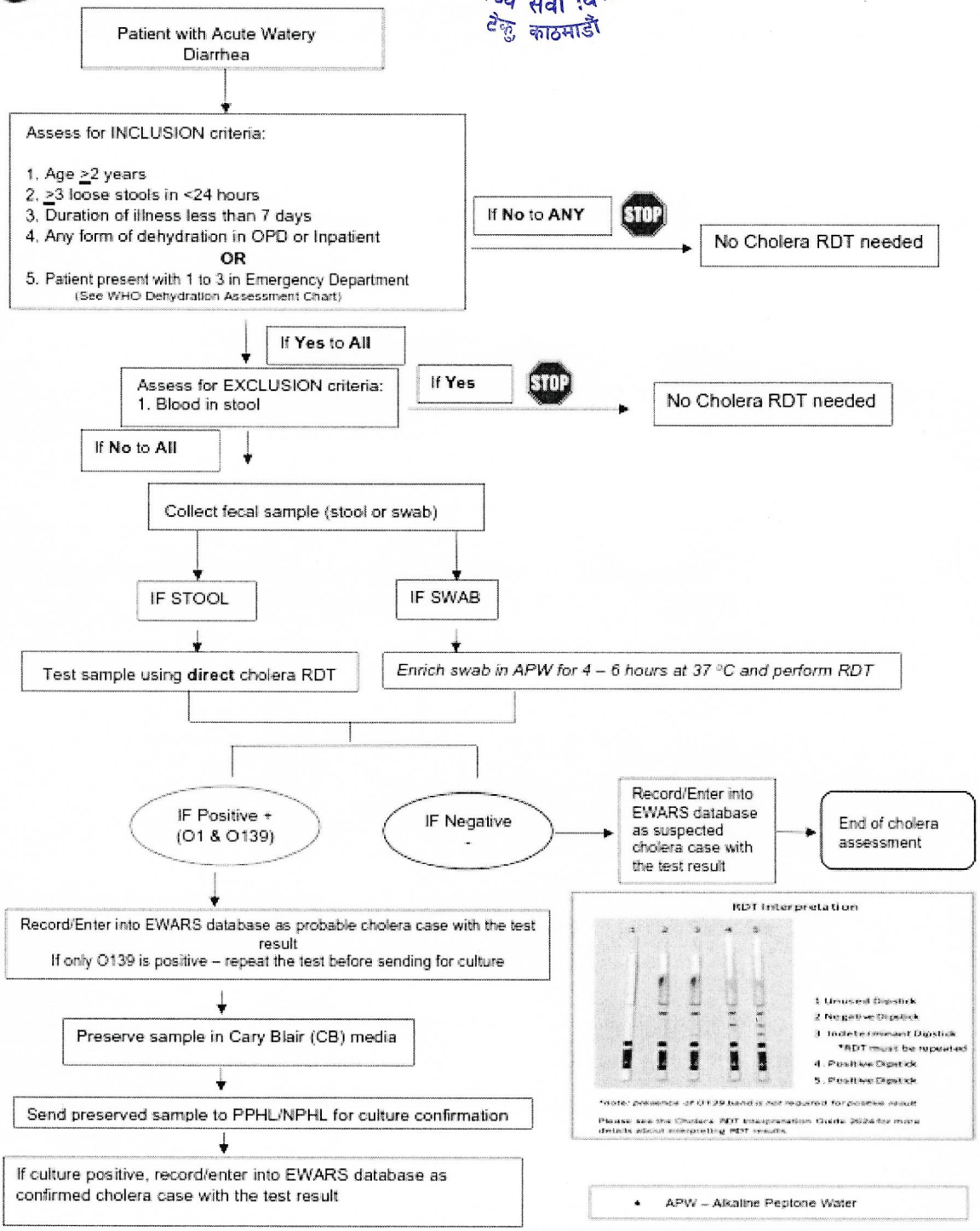
Department of Health Service
Epidemiology and Disease Control Division

Algorithm for RDT based Cholera Surveillance



Note: Sample shall be transported within 7 days for culture/PCR test and culture/PCR test shall be performed within 4 days of receipt of sample

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5. Interpretation of result

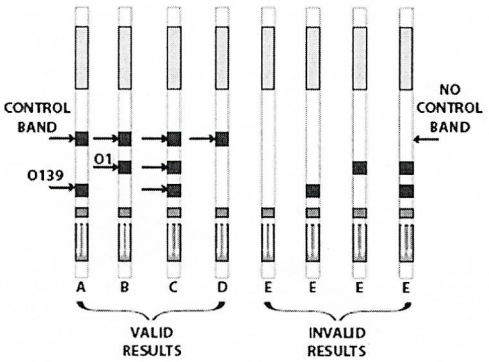
RDT test results should be interpreted as per the interpretation guide poster provided to help read the bands on the RDT dipstick for **both O1 and O139 lines** as well as based on the provided job aid poster, and lab flow charts for direct RDTs and enriched RDTs.

Results Interpretation

Interpretation*	Pinkish red band observed		
	O1	O139	Control
A. *Vibrio cholerae* O139 detected	-	+	+
B. *Vibrio cholerae* O1 detected	+	-	+
C. *Vibrio cholerae* O1 and O139 detected	+	+	+
D. *Vibrio cholerae* O1 and O139 not detected	-	-	+
E. Invalid results	+ or -	+ or -	-

* Control band must appear for the result to be considered valid.

Schematic Interpretation of Results



https://www.cdc.gov/cholera/pdf/crystal-vc-eng-p.pdf

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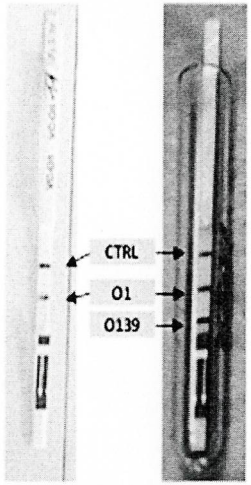
Cholera RDT Interpretation Guide 2024

Positive RDT



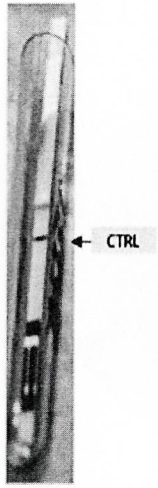
Interpretation:
Valid RDT test, possible presence of V. cholerae.

Control band is strong, There is a strong band where O1 test band should be. Positive RDT.

Next Steps: Specimen is RDT positive, send for culture/PCR confirmation.

**note: presence of O139 band is not required for positive result*

Negative RDT

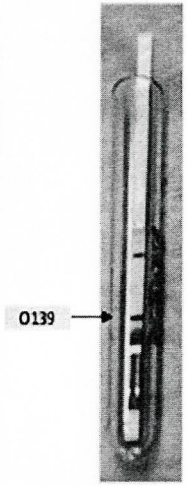


Interpretation:
Valid RDT test, negative for V. cholerae.

Control band is strong, and there is no band where O1 test band should be. Negative RDT.

Next Steps: Specimen is RDT negative, cholera investigative procedure stops here.

O139 band is present without O1 band

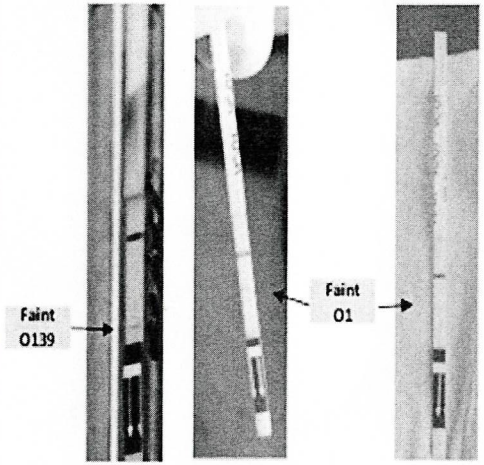


Interpretation:
Indeterminant RDT test.

Control band is strong, but the O139 test band is present without the O1 test band. This suggests the RDT worked but needs to be repeated because O139 is extremely uncommon.

Next Steps: Direct RDT needs to be repeated. If O139 test band appears clearly without the O1 test band on the repeated RDT, send for culture/PCR confirmation.

Faint O1 or O139 band appears to be present



Interpretation:
Indeterminant RDT test.

Control band is strong, but the O1 and/or O139 test band is faint. This suggests the RDT worked, but there is not a strong positive band.

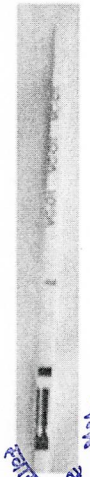
Next Steps: Direct RDT needs to be repeated for clear band.

Technical Error: RDT invalid

Interpretation: *Failed RDT test.*

1. Lack of strong positive control band
2. Discoloration of bottom tip of RDT shows that the test was not performed according to instructions. RDTs were inserted directly into the stool, turning them stool colored.

Next Steps: RDT needs to be repeated



Interpretation:
Indeterminant RDT test

Control band is present, but O1 test band is not connecting in a line across the entire width of the RDT. Only half of the test band appears.

Next Steps: Direct RDT needs to be repeated for clear band.

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6. Things to be remembered :

False negatives

False negatives from direct RDTs can occur if specimens are collected:

1) after initiating antibiotic therapy;

2) in cases of poor specimen collection or handling practices (e.g., sample collected in receptacles containing chlorine residue, extended delays between collection and testing);

3) when low numbers of bacteria are present in the sample (e.g., samples from patients that have been ill for longer than 4 days, or mild cases or suspected asymptomatic carriers).

False positives

False positivity may occur since RDT test is a screening test and requires culture/ PCR for confirmation. False positivity rates in Nepal have not surpassed 2% as evidence from the ECHO N (Enhancing Cholera Control in Nepal) surveillance (2021-2024) and RDT Implementation Strategy and Evaluation (2023-2024).

To read more: https://www.gtfcc.org/wp-content/uploads/2021/02/meeting-of-the-gtfcc-working-group-on-laboratory-surveillance-webinar-2021-amanda-debes.pdf

Use of RDT by non-EWARS sites

RDT kits will be supplied by the EDCD or PPHL to Non- EWARS sites in need or upon request. In case of outbreak, PHD and Local Authority will mobilize RRT team or perform RDT in Non-EWARS sites, in such situation PHDs will collaborate with PPHL/EDCD to avail the required RDTs. The RRTs/non-EWARS sites in return shall keep the record and report the test result to PHD/PPHL/EDCD. For the culture confirmation of the RDT positive samples, non EWARS sites will send the samples in the Cary Blair media to the PPHL or NPHL. PPHL/PHD shall record and report the number of RDT used, RDT test result and culture result of samples received from the non–EWARS sites.

Transport of samples for further testing:

If your facility does not have the capacity to verify positive results from a direct cholera RDT through culture, preserve the specimen to be sent to the PPHL/NPHL. Swabs may be stored at room temperature in Cary Blair (CB) media for 4 $\pm$ 1 days and shall reach the destination lab within 7 days.

7. Recording and Reporting RDT results:

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When you have the results from your cholera RDT, please inform your medical recorder to write *cholera RDT* (specifying direct or enriched test) in the "Lab Method" column of the EWARS reporting system. Record the result (positive or negative) under the "Lab Result" column.

If the RDT test is negative for both direct or enriched test, record and report this result as suspect cholera case in the reporting system (EWARS) as well as in the recording file / system.

If the RDT test is positive for both direct or enriched test, record and report this result as probable cholera case in the reporting system (EWARS) as well as in the recording file / system.

A sample of the recording/reporting template is in the annex.

8. Roles and Responsibilities

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Epidemiology and Disease Control Division (EDCD)

- Timely supply of RDT kits to the EWARS sites and PPHL.
- Coordination with the NPHL, PPHL, PHD and EWARS sites.
- Monitoring and Supervision of the RDT usage, data recording and reporting.
- Monitor the situation on a weekly basis and publish the data in weekly bulletin.

National Public Health Laboratory (NPHL)

- Supply of Cary Blair media for sample transportation to both EWARS and non- EWARS sites.
- Perform culture of RDT positive samples and seroagglutination for confirmation of Cholera and provide report to the sender/ EWARS Site and EDCD within 4 days of receipt of sample.
- Recording and reporting culture result to EDCD.
- Provide technical support to institute and run the Cholera Culture in PPHL.
- Coordination and Communication with EDCD, PPHL, PHD and EWARS sites.

Provincial Health Directorate (PHD)

- Coordination with the EWARS sites and PPHL
- Monitoring and Supervision of the RDT usage, data recording and reporting.

Provincial Public Health Laboratory (PPHL)

- Supply of RDT kits and Cary Blair media for sample transportation to both EWARS and non- EWARS sites as recommended by the EDCD
- Maintaining the buffer stock of RDT
- Maintain records of distribution and stock of RDT kits, perform culture of RDT positive samples for confirmation of Cholera with appropriate maintenance of records and provide immediate report to the sender/EWARS Sites and enter the same in EWARS platform.

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- Report of the RDT reserve and RDT supplied to non-EWARS site.
- Coordination and Communication with EWARS sites, EDCD, PHD
- In case of outbreak, supply the RDT kits to PHD or RRT in collaboration with PHD,
- Report the RDT kits used in outbreaks with its finding on a monthly basis.

EWARS Sites

- Using RDT kits for the suspected cholera cases.
- Maintaining the stock of RDT kits.
- Recording and reporting of RDT test result and culture result in the EWARS reporting system.
- Send the samples preserved in Cary Blair media for further confirmation by Culture or PCR to the respective lab within 7 days.
- Coordination and Communication with EDCD, PHD, NPHL or PPHL.

Supporting Partners

- Coordination and Communication with EDCD, PHD, NPHL or PPHL.
- Adopt the protocol in ongoing cholera surveillance

For further clarification, please call the Epidemiology and Disease Control Division (EDCD) at: +977014255796

For further reading: https://www.gtfcc.org/wp-content/uploads/2024/04/public-health-surveillance-for-cholera-guidance-document-2024.pdf

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9. Annex: Reporting Template

Reporting template

Name of Health Facility:

Address:

Date of RDT used	Patient ID	Patient's Name	Age	Sex	Address	Form of Dehydration	Antibiotics prior to sample collection	Specimen type	RDT test method (Direct or Enriched)	Test result	Species identified (O1/O139)	Comfirmation method (Culture/PCR)	Final Diagnosis

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