

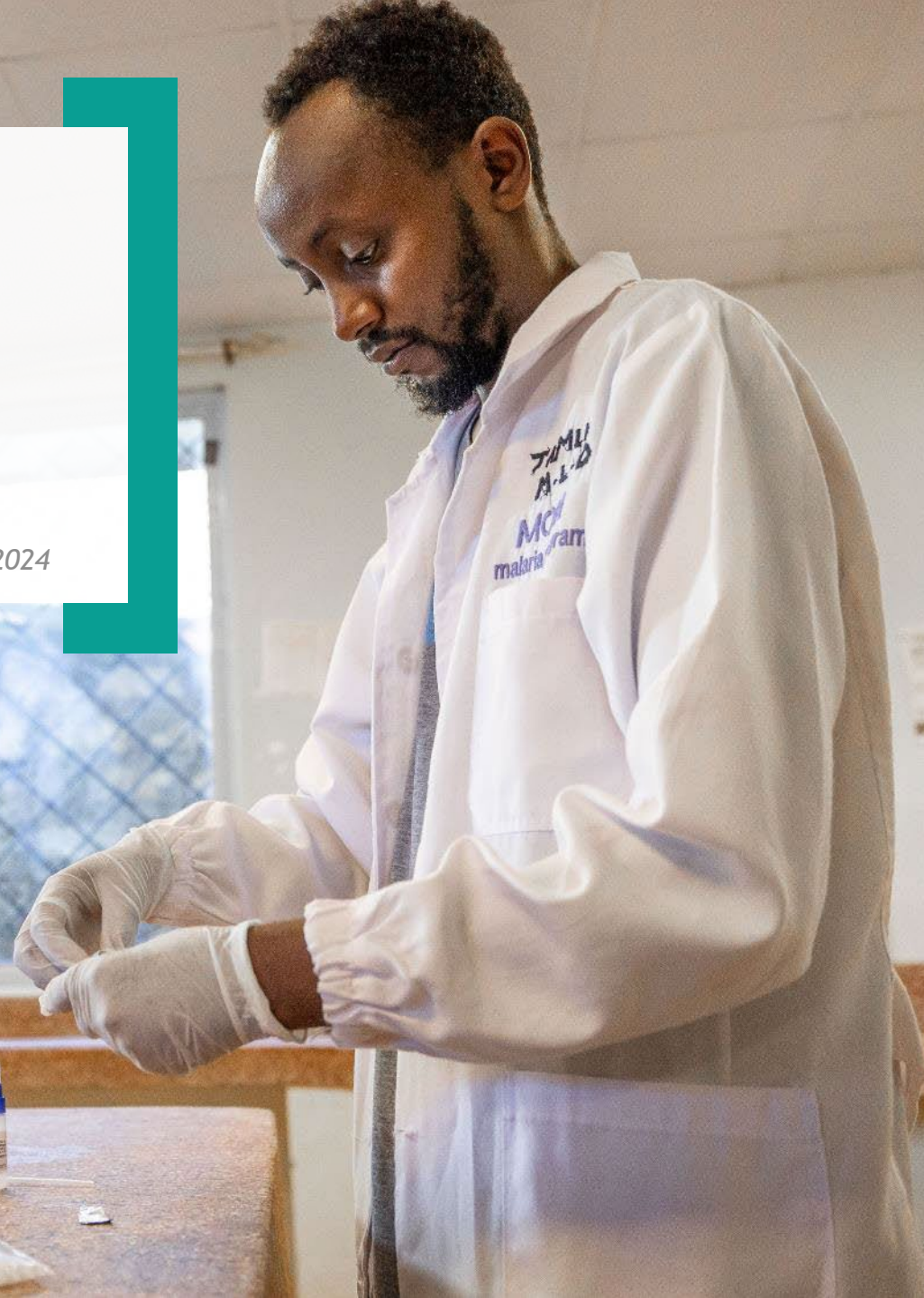


GLOBAL TASK FORCE ON  
**CHOLERA CONTROL**

GTfCC recommendations

## CHOLERA RAPID DIAGNOSTIC TESTS (RDTs)

V1.0 November 2024





# Learning objectives

- Understand what RDTs are

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- Explain when and why to use RDTs for cholera

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- Understand the limitations of RDTs for cholera

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- Know the procedure to perform RDTs for cholera

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- Interpret RDT results

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- Troubleshoot RDTs (procedure and results)

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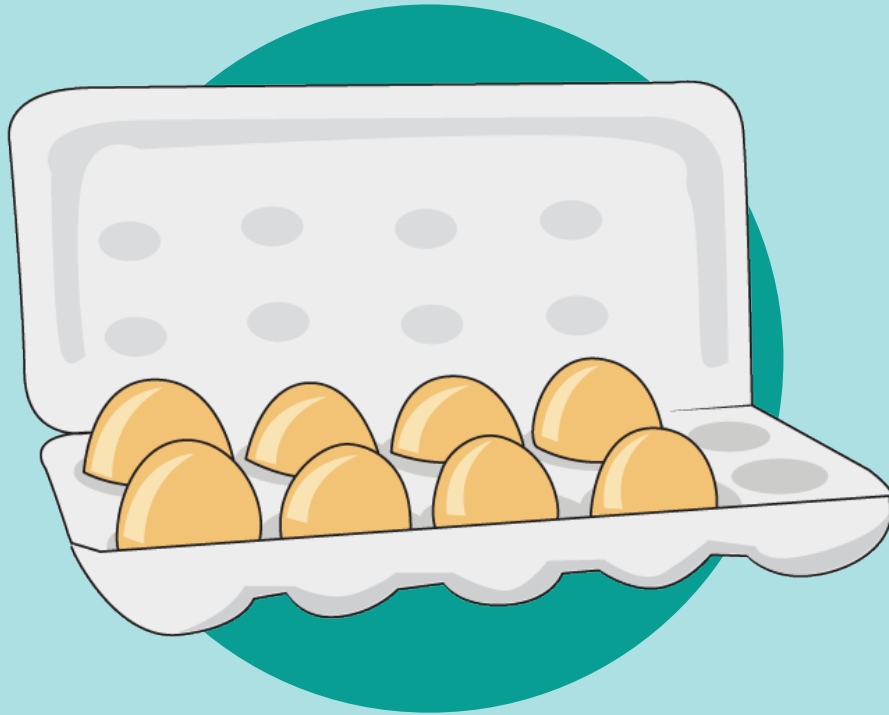
**Suggested previous modules:** module 1 Introduction to Cholera and Cholera testing



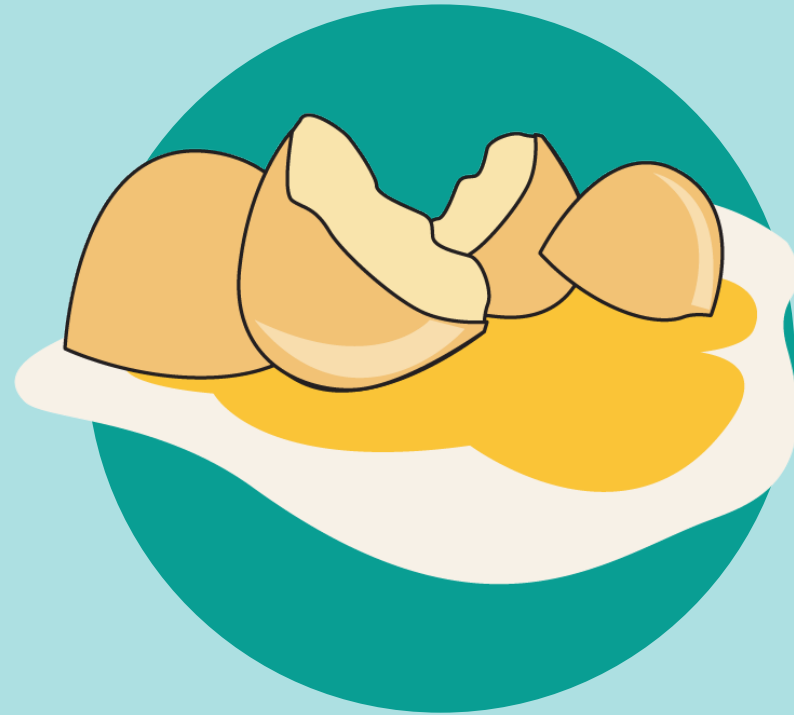
# Outline

- 1 RDTs: what, why, when
- 2 Safety
- 3 Procedure
- 4 Reading the results
- 5 Reporting
- 6 End of module assessment

“ *The result of any laboratory examination is only as good as the sample received in the laboratory.* ”



*Good sample*



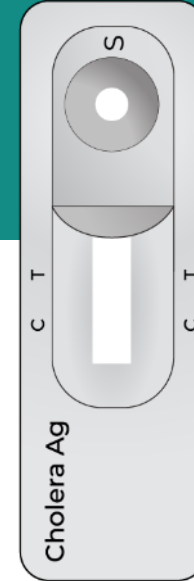
*Bad sample*



# WHAT • WHY • WHEN

## RDTS -

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# What are RDTs?

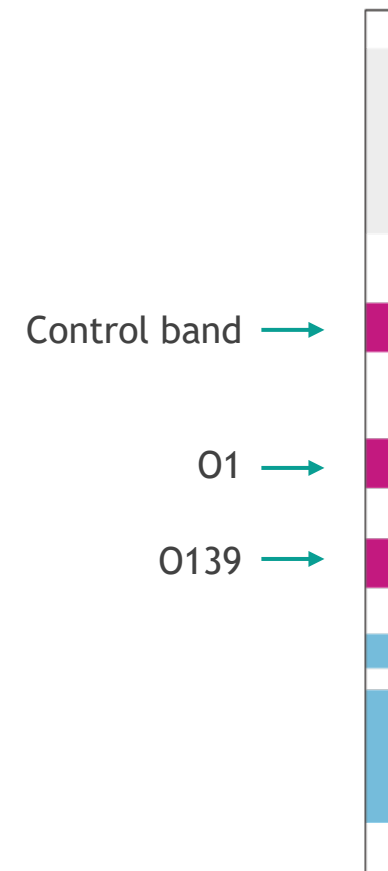
## Concept: *Lateral flow immuno- chromatographic assays*

- Rapid detection of O1 or O1 and O139 antigens (<30min)
- Direct detection in stool samples
- Do not require complex laboratory set up

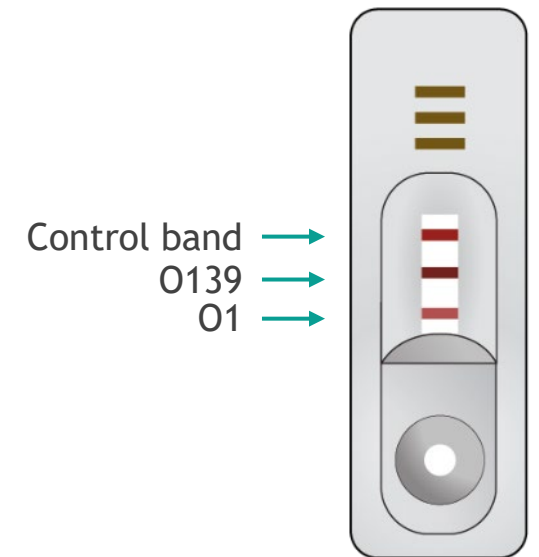
## Packaging:

- Cassette
- Dipstick  
*(just like home pregnancy tests or COVID tests)*
- Packaged as kits including all reagents needed

Dipstick



Cassette



# Products available

There are multiple commercially available RDTs with varying levels of accuracy making many of them less appropriate for use.

None of them are WHO pre-qualified as of yet.

WHO  
recommends  
and distributes:



Crystal VC Ag 01/O139  
(Kit with 10 tests)



SD Bioline Cholera Ag 01/O139  
(Kit with 20 tests)

# Why use RDTs?

- ▶ Usable at the « bed-side » (need to collect stool sample)
- ▶ Rapid: results in < 30 minutes
- ▶ Inexpensive
- ▶ Easy to use
- ▶ No sample storage or transport



# Why use RDTs?

- RDTs are used :
  - In the context of **absence of a probable or confirmed outbreak**, to speed up the detection of a **suspected cholera outbreak** or a **probable cholera outbreak**
  - To monitor a **probable or confirmed outbreak (community transmission)**
- The strategy to reach each of these objectives is described in the GTFCC recommendations for [Public Health Surveillance for Cholera](#).



# Summary

|                                   | No outbreak   | Outbreak  |
|-----------------------------------|---|---|
| 1 Treat the patient               | Treat all, rehydration protocols (ATB)  |   |
| 2 Identify a cholera suspect case | ≥ 2 yo, with AWD and severe dehydration, or died from AWD                       | Any age, with AWD or died from AWD  |
| 3 Test for cholera                | RDT on all suspect cases<br>Collect and send sample from all RDT+ to laboratory | RDT on first 3 suspected cases of the day<br>Collect and send 3 RDT+ samples per week from the area to laboratory |
| 4 Document and report             | Record immediately<br>Report daily on RDT+                                      | Record immediately<br>Report weekly   |

# Samples for RDTs

RDTs should ideally be performed on fresh stool specimens **within 2 hours of collection** from a patient who has been **ill for less than 4 days** and who has **not been given antibiotics**.

▼ **Appropriate** stool specimens for direct testing by RDTs:

- liquid stool
- viscous, mucoid, soft or semi-solid stool

▼ **Inappropriate** stool specimens for direct testing by RDTs:

- stool preserved in Cary-Blair transport medium
- rectal swabs

**Note:** These specimens require additional steps (for example, enrichment) in a laboratory.

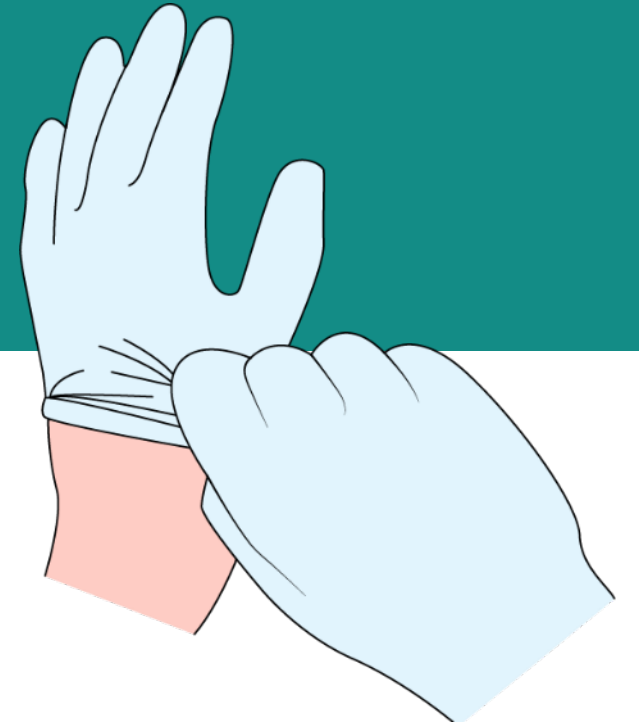


## RDTs - Limitations

- Cholera RDTs are **not a replacement for stool culture or any molecular test** to confirm cholera or to confirm a cholera outbreak.
- Cholera RDTs **cannot be used for diagnosis of individual cholera cases.**
- The result of cholera RDTs **should not influence case management.**
- Cholera RDTs **do not detect the cholera toxin.**
- Cholera RDTs **do not provide antimicrobial susceptibility data.**



**SAFETY**



## Basic hygiene practices

# Safety first

Protect yourself, your patients and your community.



If you have cuts or abrasions on the skin of your hands, cover them with adhesive dressing.



Wear gloves when collecting and handling stool specimens.



Remove gloves and wash your hands after completing any task involving the handling of stool specimens.



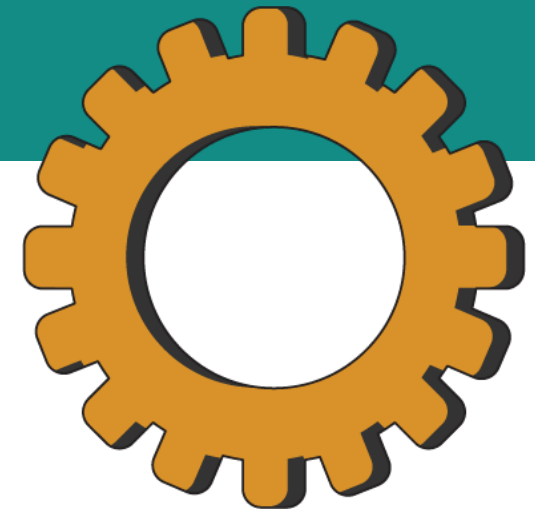
Adhere to proper waste disposal procedures

You can also protect your clothes by wearing scrubs or a lab coat.





PROCEDURE





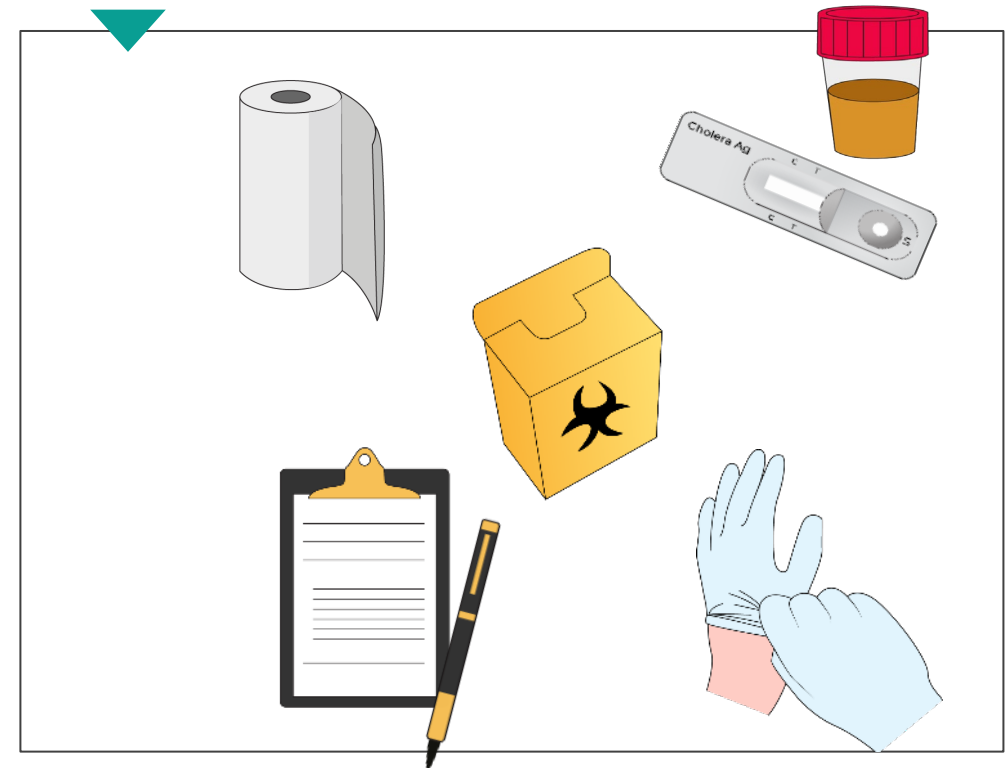
# Considerations prior to testing

- Consider the testing strategy applied currently in your area (suspect case definition, strategy, who and when to test).
- Verify expiry dates of RDTs on hand.
- Verify adequate storage conditions of RDTs as per manufacturer's instructions.
- Read the manufacturer's instructions for use.
- Allow all kit components and specimens to reach a temperature of 15°C-30°C before testing.
- Procedure may vary and include a sample enrichment step in Alkaline Peptone Water (APW).

# Supplies and material

RDTs should be performed on a stable surface that can easily be cleaned.

Gloves and scrubs/labcoat  
Hazardous waste bin or bag  
Tissue paper  
Test kit  
Timer  
Register/forms  
Marker/pen





SOP

# Crystal<sup>®</sup> VC 01/O139, Arkray

## Dipstick method

1

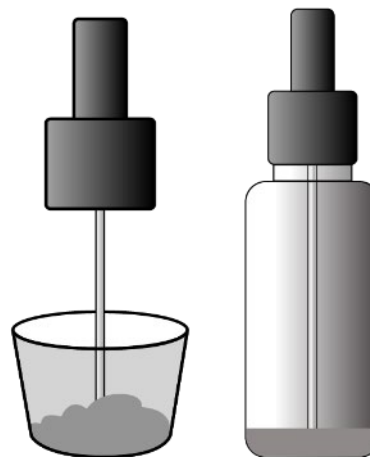
Label tube with patient identifier. Open the cap of the sample processing vial.



Sample processing vial

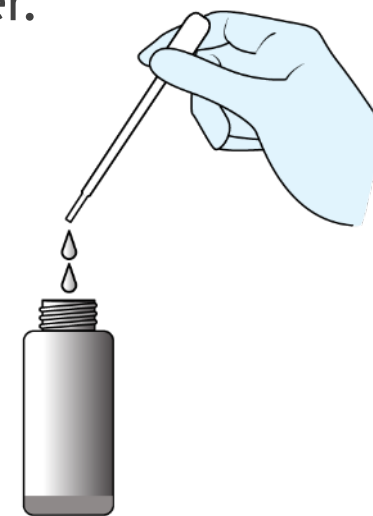
2

**Semi-solid faecal specimens:** Collect a small quantity of stool using the collection stick and transfer to the tube containing reaction buffer.



or

**Liquid faecal specimens:** Draw liquid specimen using the dropper and transfer 2 drops to the tube containing reaction buffer.

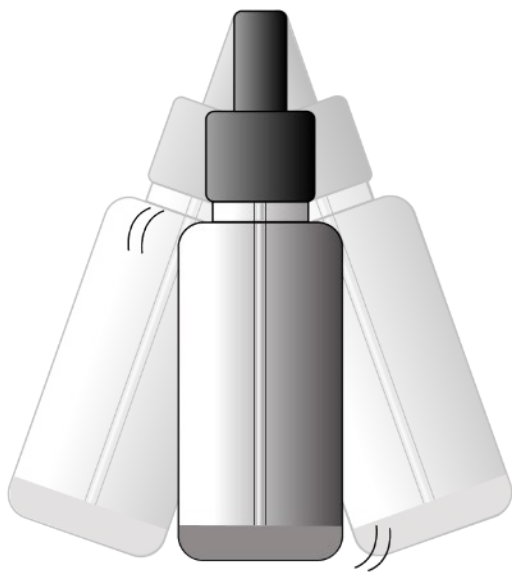


Discard the dropper in the sharps container or double-lined plastic bag labelled “biohazard” after adding specimen.



3

Tightly recap sample processing vial and shake to mix contents.



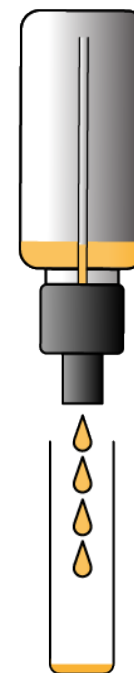
4

Break open the outer end of the cap (point away and cover with tissue to avoid splash).



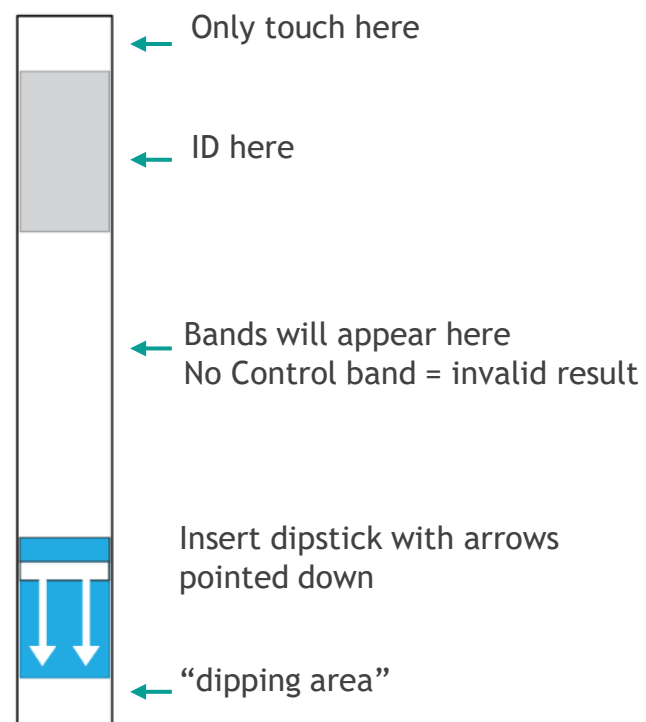
5

Dispense 4 drops of processed sample into labelled 5ml test tube.

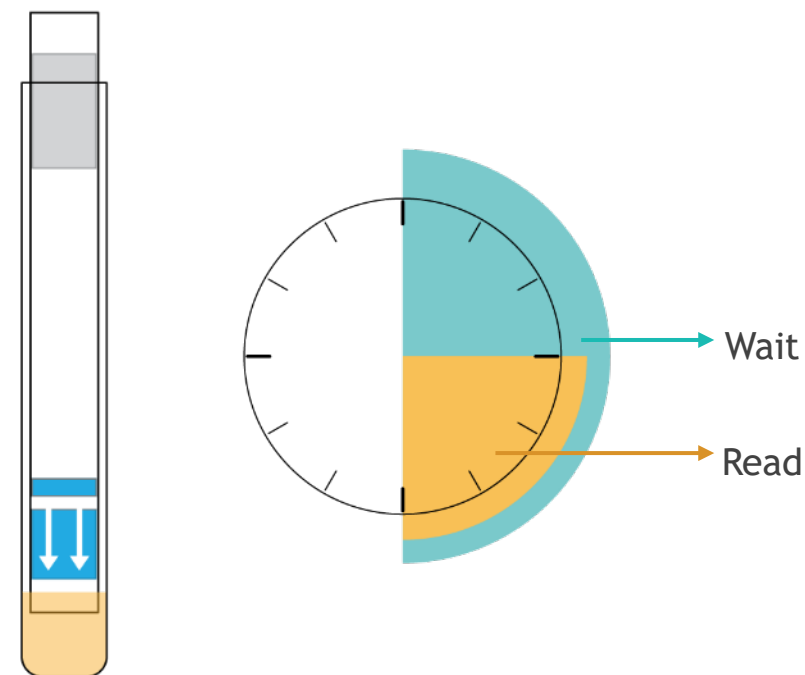




Carefully open test pouch. Discard if damaged, or if desiccant is missing or changed in colour. Write patient ID on the dipstick.



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





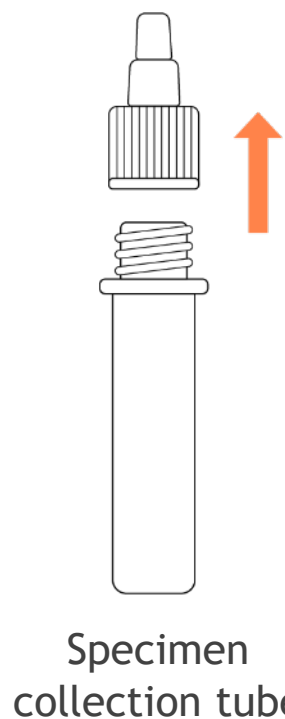
SOP

# Bioline™ Cholera Ag O1/O139, Abbott

## Cassette method

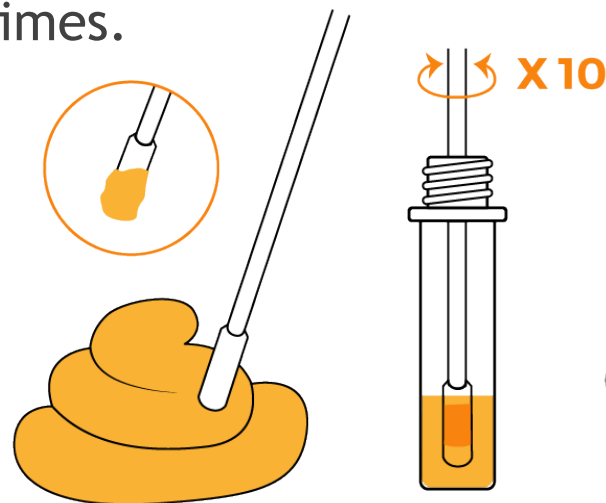
1

Label tube with patient identifier. Open the cap of the specimen collection tube.

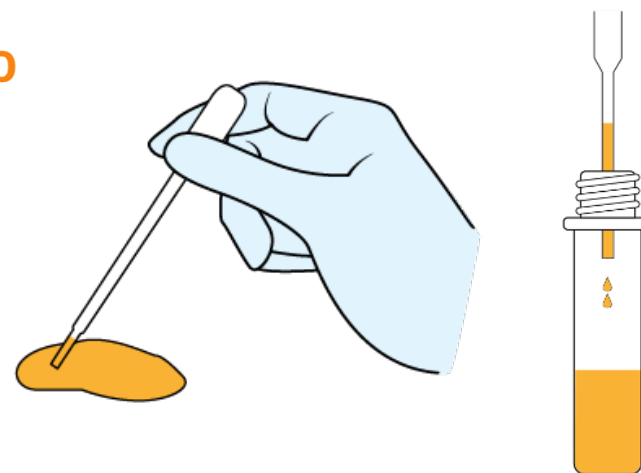


2

**Semi-solid faecal specimens:** Collect sufficient specimen using the collection swab and insert the swab into collection tube and swirl the swab at least 10 times.

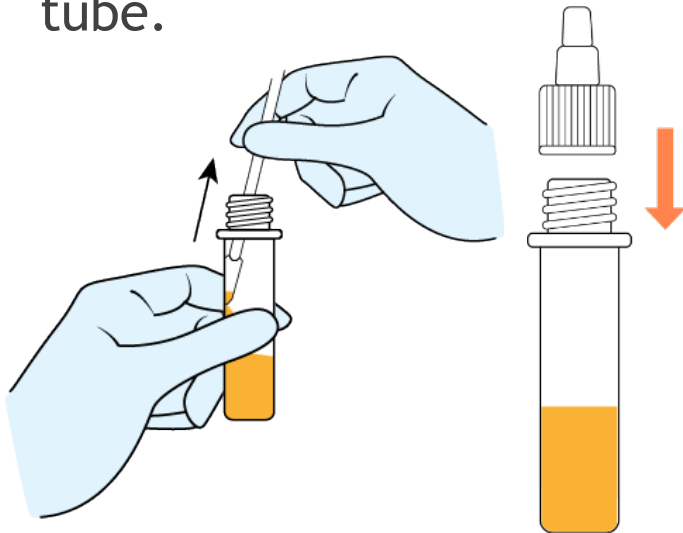


**Liquid faecal specimens:** Draw liquid specimen up to the fill line using the dropper and transfer to the tube containing reaction buffer

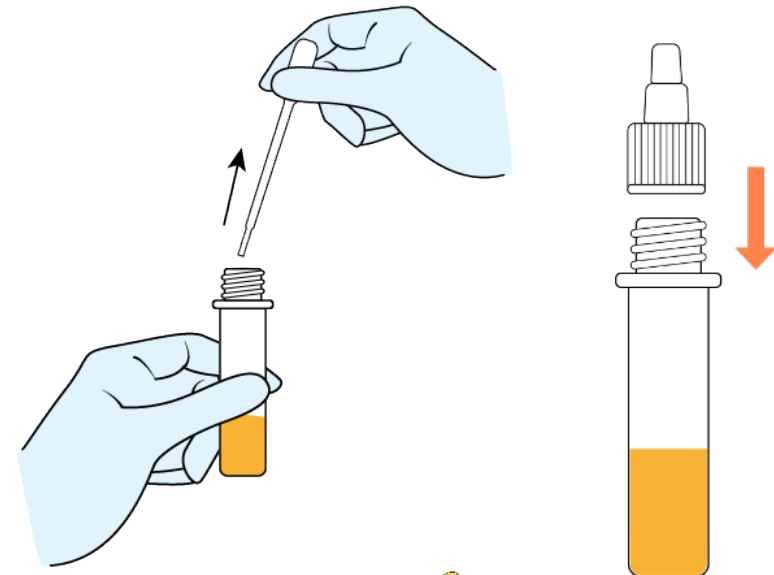


**Semi-solid faecal specimens:**

Remove the swab while squeezing the swab against the wall of tube. Assemble the filter cap on the specimen collection tube.

**Liquid faecal specimens:**

Discard the dropper. Assemble the filter cap on the specimen collection tube.



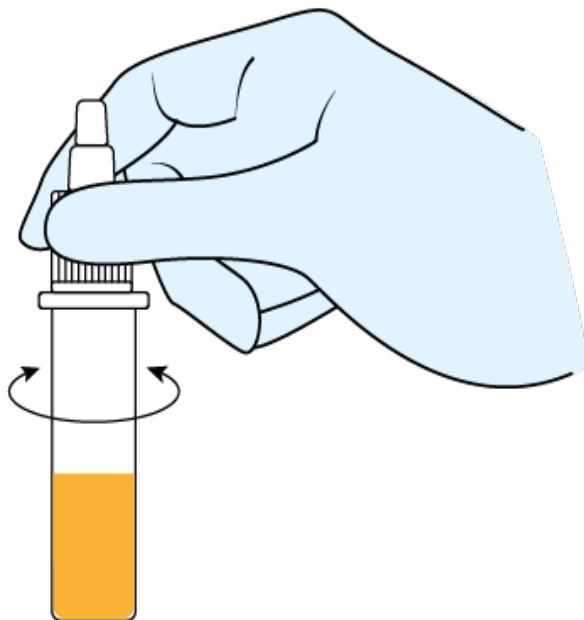
or

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



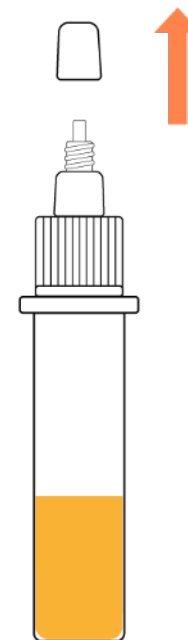
4

Shake tube thoroughly to ensure proper mixing of the fecal specimen with extraction buffer.

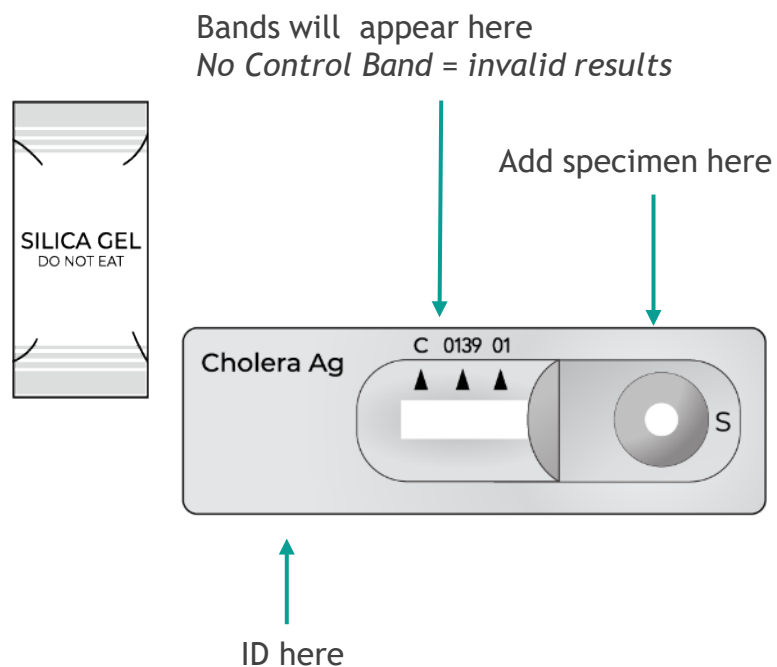


5

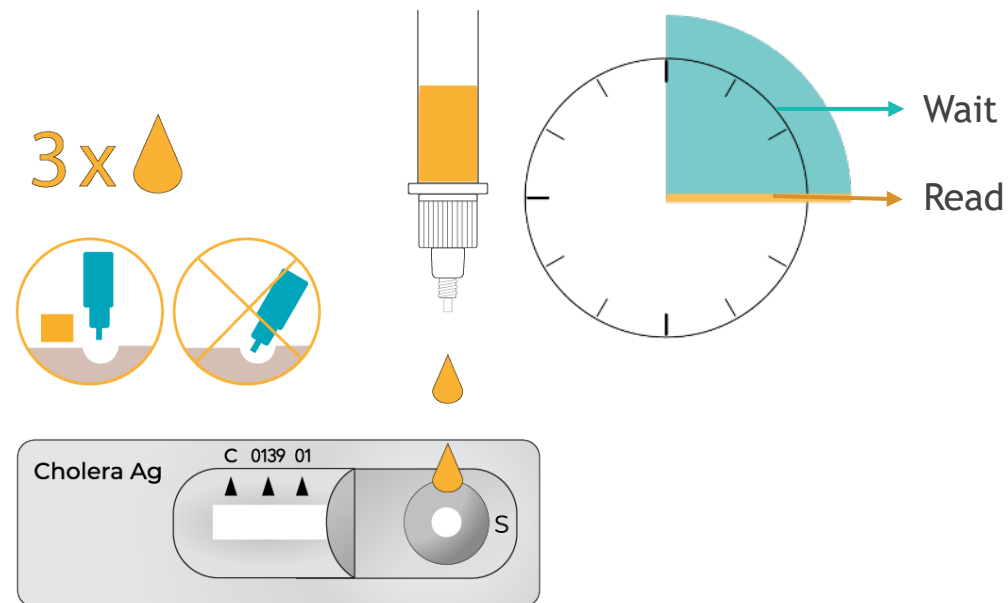
Unscrew the nozzle cap of the specimen collection tube.



Carefully open test pouch. Discard if damaged, or if desiccant is missing or changed in colour. Write patient ID on the cassette.




Hold the collection tube vertically and dispense 3 drops into the specimen well "S".








# Troubleshooting the procedure




RDTs not stored according to manufacturer's recommendations or out of date.



Manufacturers may have changed the procedure.



Too much or too little sample used.



Failure to check kit contents prior to performing the test.

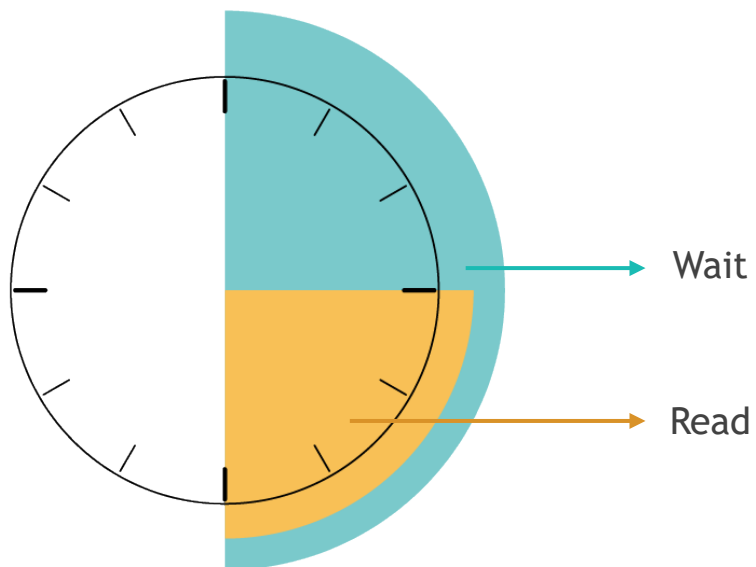


# READING THE RESULTS

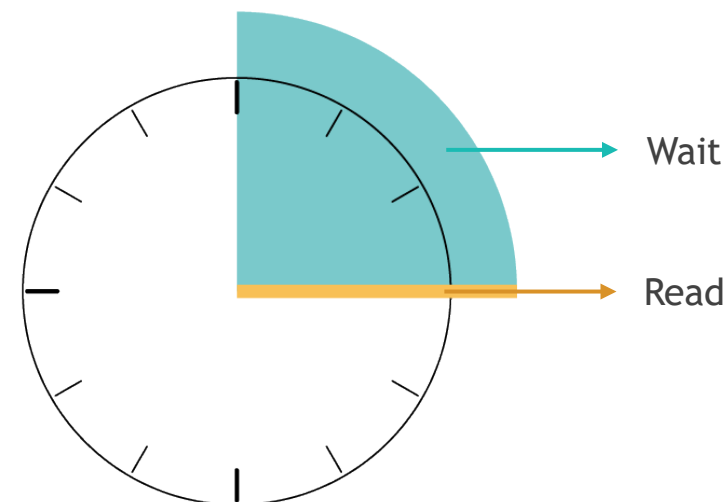


# Waiting to read the results

**Dipstick:** Wait 15-30 minutes then hold the test-tube and raise the strip up so you can read the bands but do not remove the bottom of the strip from the tube to avoid making a mess.



**Cassette:** Wait 15 minutes and read results immediately.  
Do not read after 15 minutes.



Use a laboratory timer or the timer on your phone for correct reading time.

# Results: Reactive / non-reactive

## Reactive

- The sample reacted with the test lines
- A line is seen in the testing area for VC O1 or VC O139
- Indicates a strong probability of cholera infection but is not confirmatory
- "Positive" is reserved for confirmed cases after laboratory testing.

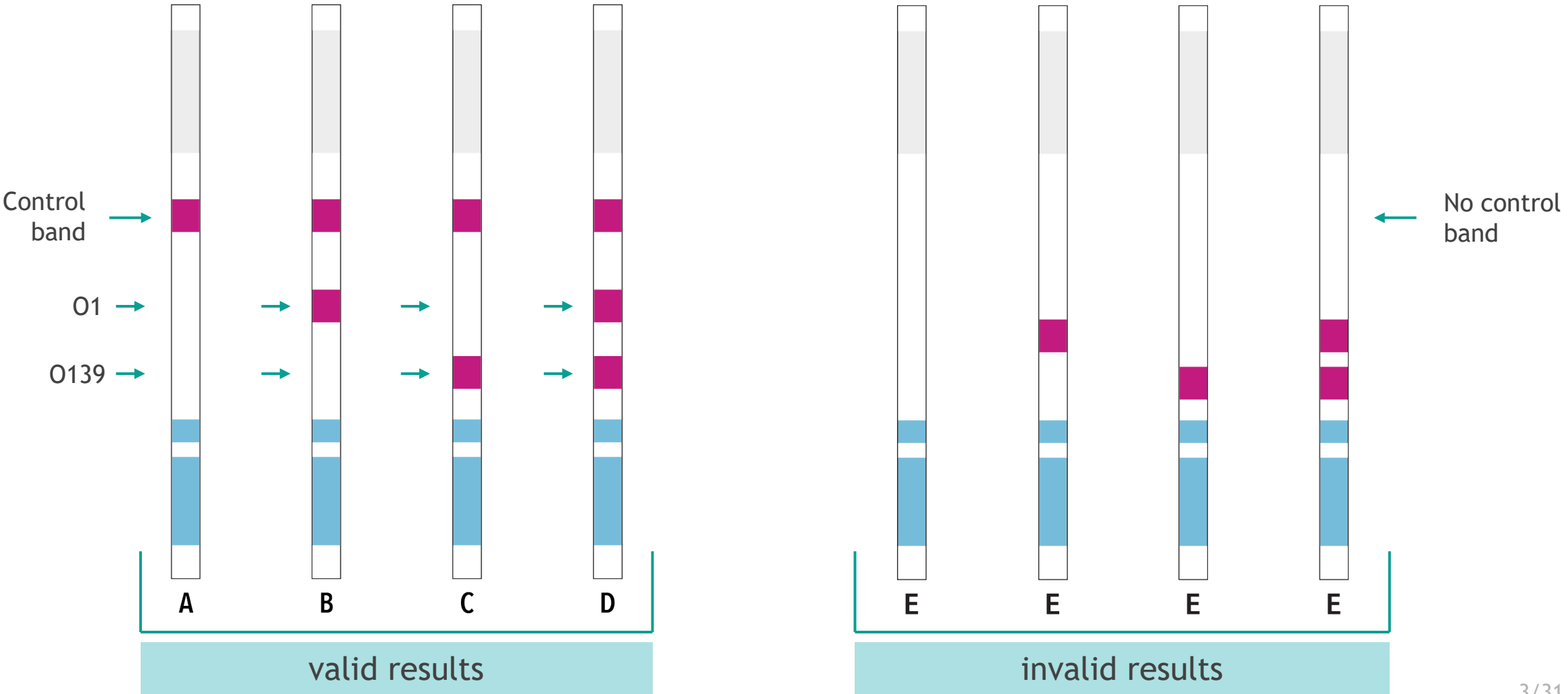
## Non-reactive

- The sample does not react with the test lines
- No lines are seen in the testing area
- Does not rule out cholera completely
- "Negative" can imply certainty, which the RDT cannot provide.

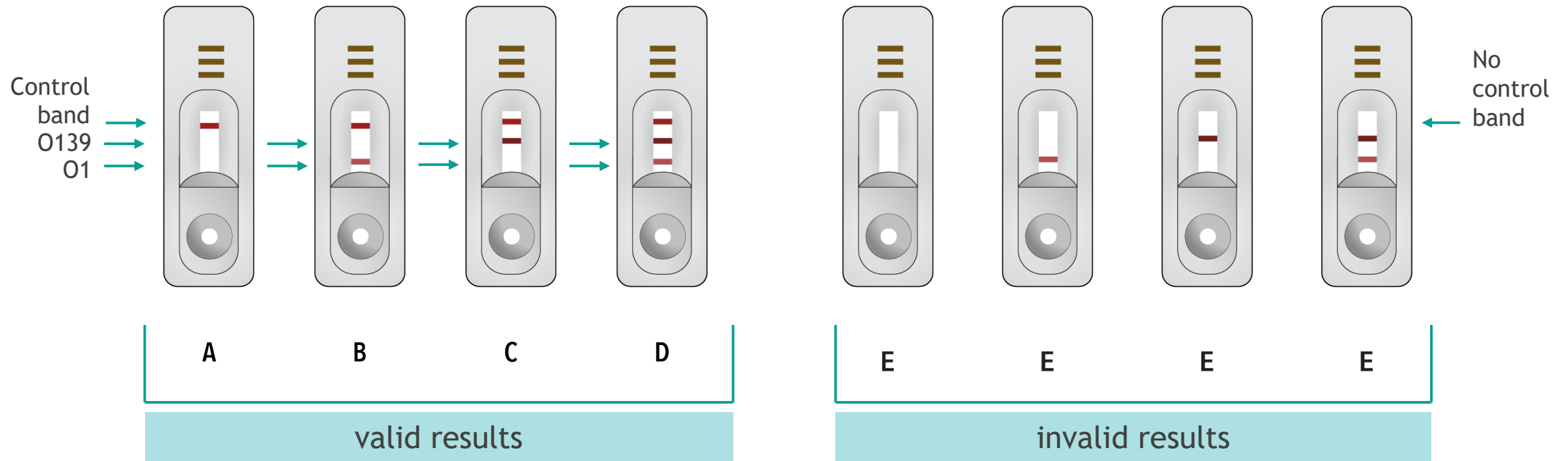
Clear terminology ensures proper case management and public health action.



# Results: Dipstick




# Results: Cassette









# Key recommendations for reading of results



Each RDT test kit, even from the same manufacturer, may have different positions for test and control bands/lines on the test. Read the instructions provided with the specific RDT in use for correct reporting.



If the control line does not appear, the test is invalid and the test should be repeated.

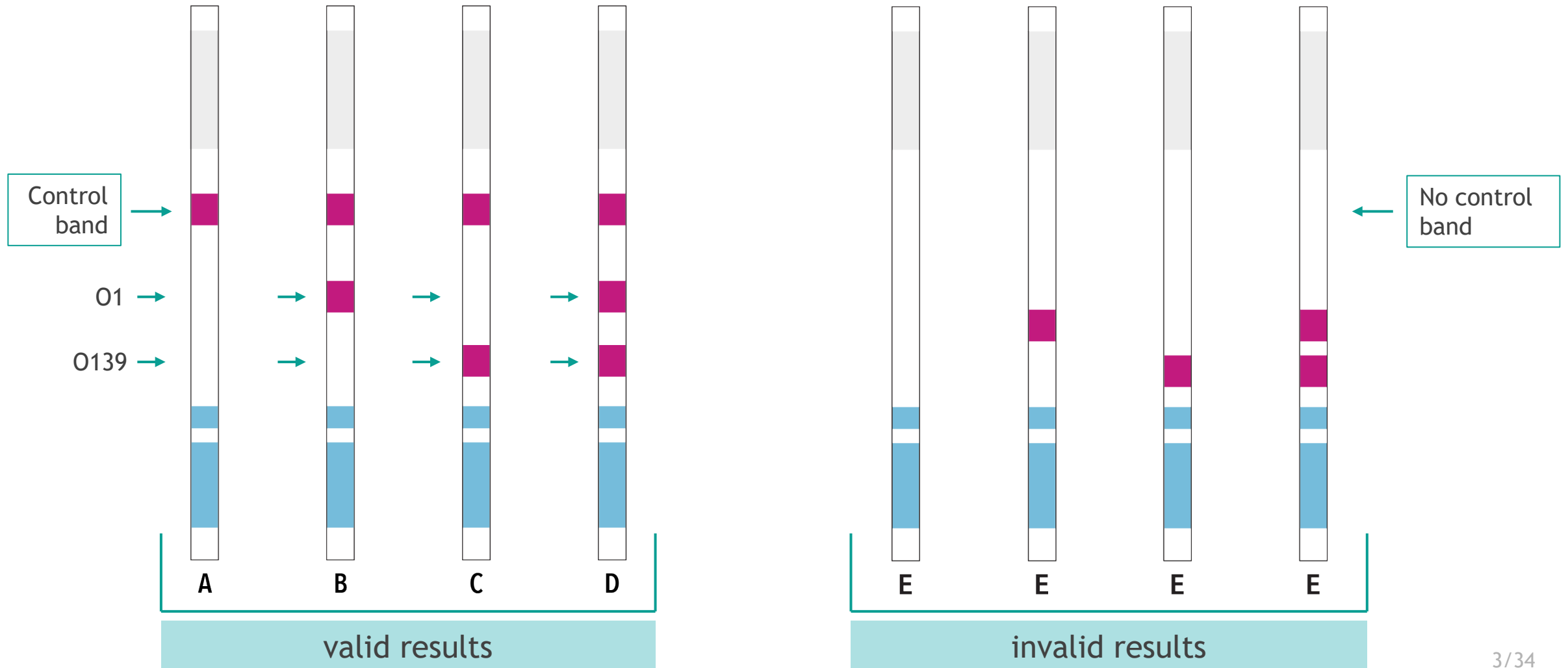


Even a faint / weak test band is considered to be reactive.



# Control band

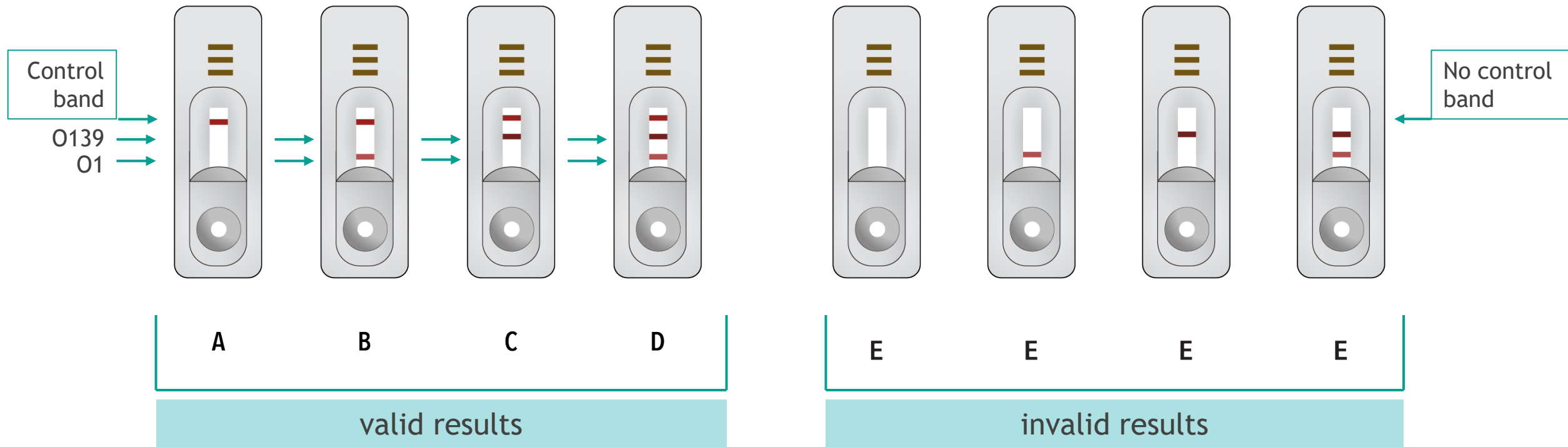
The control line **MUST** appear for a test to be valid. If it does not appear, the result is considered invalid, and the specimen should be rettested using a new test kit.





# Control band

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

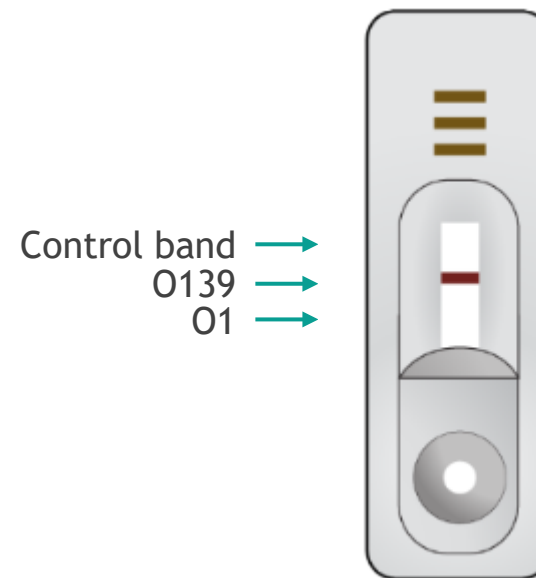
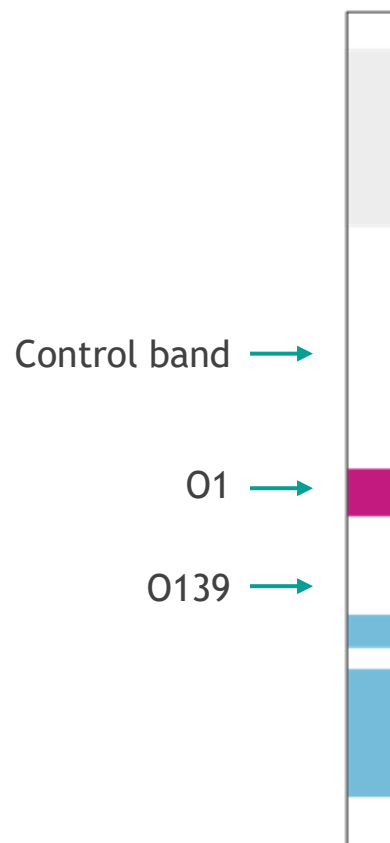


# Invalid: reading

- No visible control band

## ACTIONS

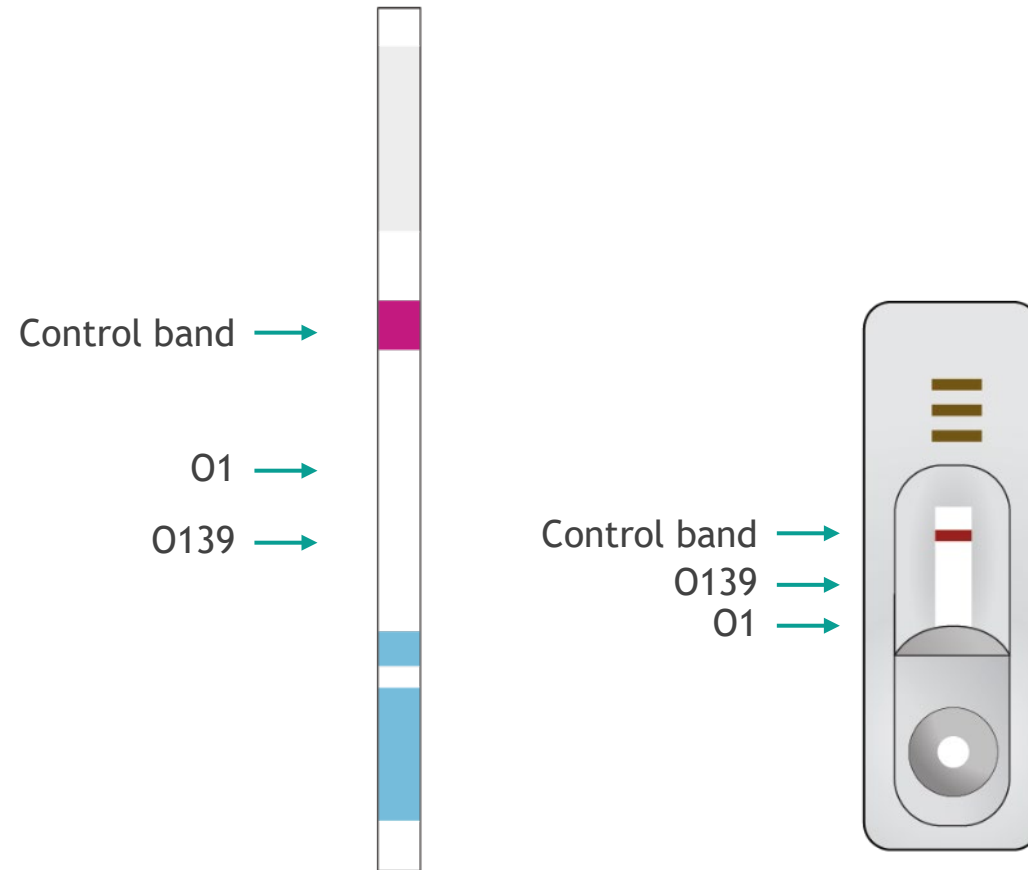
1. Record RDT invalid
2. Redo the test
3. Report final result



# O1 and O139 non-reactive: reading

- Visible control band
- No band for O1
- No band for O139

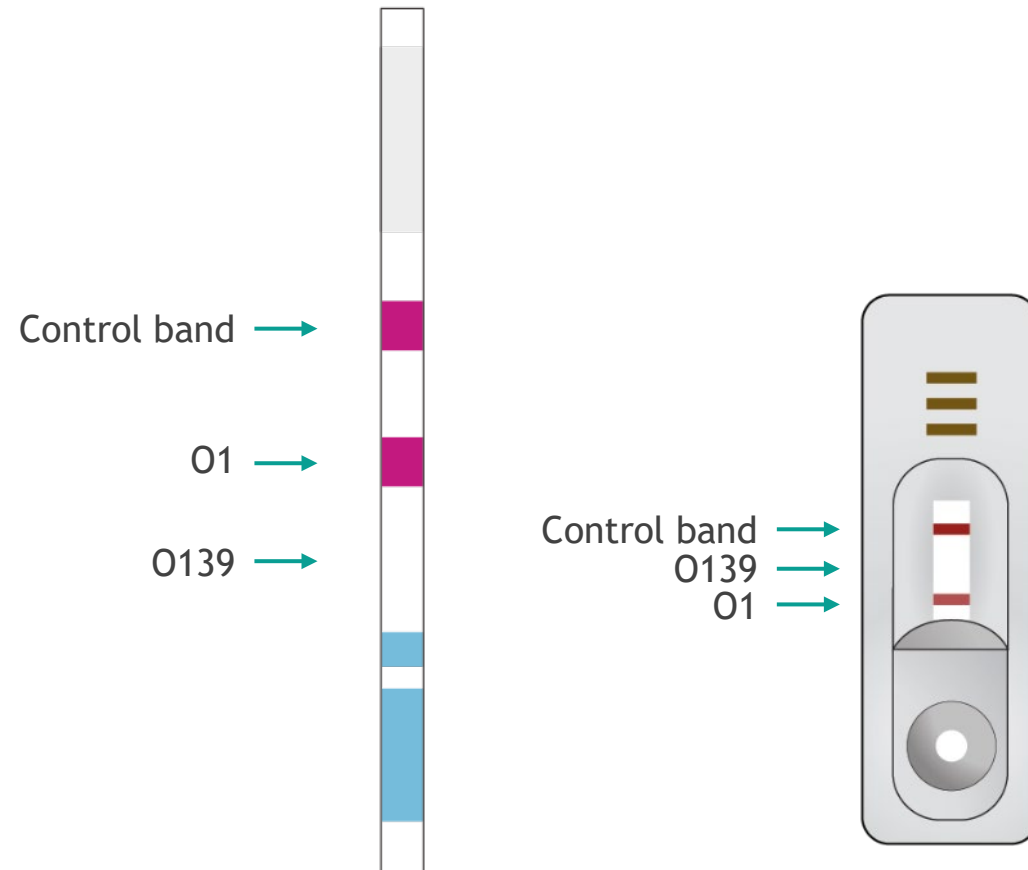
**ACTION**  
Report RDT  
non-reactive  
result



# O1 reactive: reading

- Visible control band
- Visible O1 band
- No O139 band

**ACTION**  
**Report RDT**  
**reactive for O1**



# O139 reactive: reading

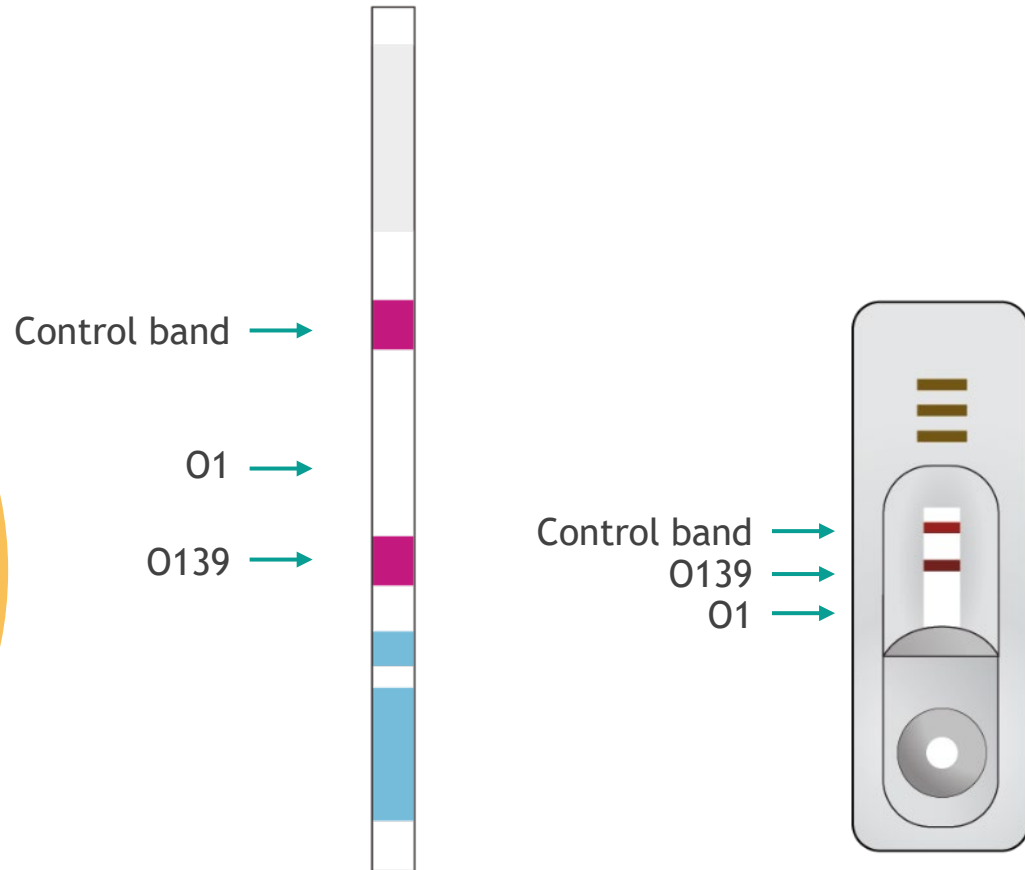
VC O139 does not currently circulate outside of South East Asia.

Current tests do not always perform well for O139, sometimes the O139 band might falsely appear.

- Visible control band
- Visible O139 band
- No O1 band

## ACTIONS

- 1.Redo the test
- 2.If same results, report reactive O139
- 3.Send the sample to a laboratory for further confirmation



# O1 and O139 reactive: reading

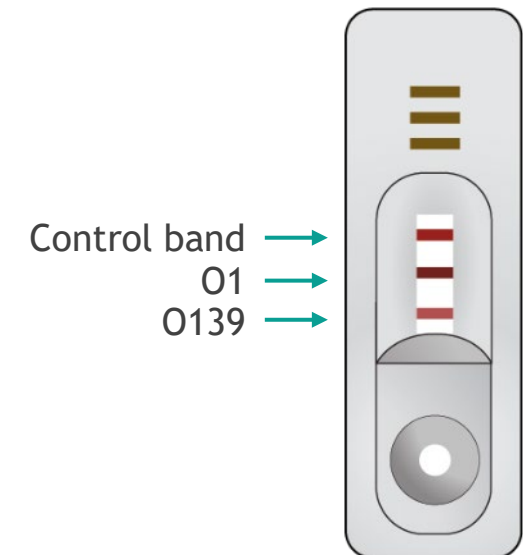
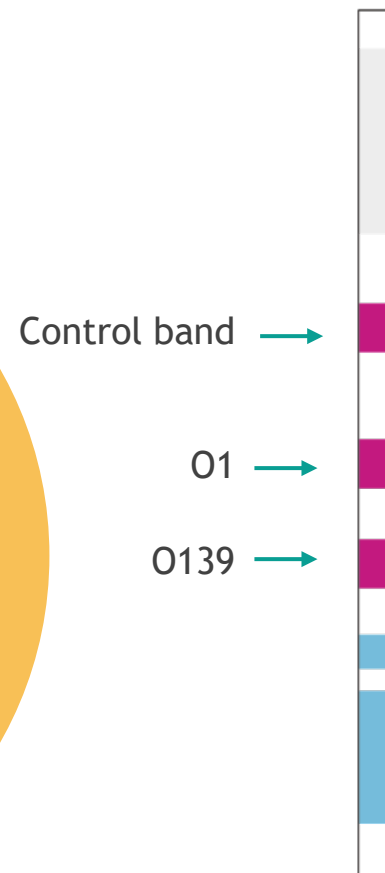
O139 does not currently circulate outside of South Asia.

Current tests do not always perform well for O139 and sometimes the O139 band might falsely appear.

- Visible control band
- Visible O1 band
- Visible O139 band

## ACTIONS

- 1.Redo the test
- 2.If same result, report O1 and O139 reactive
- 3.Send the sample to a laboratory for further confirmation





# Troubleshooting the results

Results not read in the recommended timeframe (waited too long or too little).

Misinterpretation of results.  
Misreading of weak bands.

The absence of a control band invalidates the test, which should therefore be repeated.

False negatives/  
false positives can occur.

- **RDT reactive** = strong probability that a suspected case is infected with VC but not 100% certainty
- **RDT non-reactive** = strong probability that a suspected case is not infected with VC but not 100% certainty



# REPORTING





Any and all RDT results should be immediately recorded in a electronic or paper-based register.



Any and all RDT results should be reported, even if the RDT is non-reactive or invalid.



# Reporting RDT results

- **Who to report to:** local health authorities and the laboratory if the sample is being sent there.
- **Why:** health authorities need the RDT results to take action and adapt the response to an outbreak ; the laboratory takes into consideration the RDT results during their own testing.
- **What to report:** patient and sample information and RDT result.
- **How:** RDT results must be reported in the [case report form](#) and if a sample is sent to a laboratory, the RDT results must also be reported on the [sample referral form](#).




# Key recommendations for reporting

- ▶ Write and check the patient ID/sample ID so results can be matched to a person
- ▶ Saying you have no information is also information
- ▶ Reporting O139? Pause and reflect on the situation



Did you  
**redo** the  
test?

# GTFCC Laboratory referral form for cholera suspected case

 **GLOBAL TASK FORCE ON CHOLERA CONTROL**

## GTFCC Laboratory Referral Form for Cholera Suspected Case

The referring health worker is to complete this form and send a copy to the laboratory with the specimen (one form per specimen sent).

Please attach a copy of the [Certificate and Invoice form](#).

For specific instructions for packaging and transportation please refer to [Specimen Packaging and Dispatch of Specimen for Laboratory Confirmation of Cholera Disease \(WHO 2013\)](#).

**Request made by** \_\_\_\_\_

Name of health facility (or name of health facility identified) \_\_\_\_\_

Date of request: DD / MM / YYYY \_\_\_\_\_

Name of referring health worker: \_\_\_\_\_

Address: \_\_\_\_\_

Phone: \_\_\_\_\_ E-mail: \_\_\_\_\_

**Request made for** \_\_\_\_\_

☐ Laboratory confirmation of Cholera ☐ Antimicrobial Susceptibility Testing ☐ Other, specify: \_\_\_\_\_

**Specimen** \_\_\_\_\_

Specimen ID: \_\_\_\_\_ Date and time of collection: DD / MM / YYYY Hour / Minute \_\_\_\_\_

Location specimen collected: \_\_\_\_\_

Type of specimen collected: ☐ Stool ☐ Rectal swab ☐ Other, specify: \_\_\_\_\_

Blood observed in stool: ☐ Yes ☐ No

Appearance of specimen: ☐ Brown ☐ Soft ☐ Watery ☐ Bloody mucus

Conditioning of stool sample: ☐ Stool in container (no added reagents) ☐ In Cary Blair ☐ In alkaline / phosphate buffer (DIFW) ☐ no filter paper ☐ other, specify: \_\_\_\_\_

Date specimen sent to referral laboratory: DD / MM / YYYY \_\_\_\_\_

If date of specimen collection and date specimen sent are different, how was the specimen stored (specify temperature)? \_\_\_\_\_

Was an RDT performed on the same specimen? ☐ No ☐ Yes, specify: ☐ Smeared RDT ☐ Direct RDT

Result: ☐ Reactive ☐ Reactive O139 ☐ Reactive O1 and O139 ☐ Non-reactive ☐ Invalid

Name of RDT kit used: \_\_\_\_\_

1. A line must be drawn across the top of the form and the name of the health facility must be written in the space provided.

Page 1 of 2 (5/2013) GTFCC Form

**Patient** \_\_\_\_\_

Last name: \_\_\_\_\_ First name: \_\_\_\_\_

Patient ID: \_\_\_\_\_ Sex: ☐ Male ☐ Female

Age: \_\_\_\_\_ Years \_\_\_\_\_ Months \_\_\_\_\_ Days \_\_\_\_\_ Date of birth: DD / MM / YYYY \_\_\_\_\_

Date of onset of illness: DD / MM / YYYY \_\_\_\_\_

Where did the patient first feel sick? Region? \_\_\_\_\_ District: \_\_\_\_\_ Town: \_\_\_\_\_

Patient outcome at time of request: ☐ Hospitalised ☐ Discharged ☐ Deceased ☐ Self-discharged ☐ Recovered, healthy ☐ Unknown

Antibiotics treatment received prior to collection of sample? ☐ No ☐ Yes

Any antibiotics received by the patient prior to sample collection (any, negatively impact laboratory results)? \_\_\_\_\_

Specify antibiotic(s) used: \_\_\_\_\_ Specify duration of treatment (days): DD / MM / YYYY \_\_\_\_\_

Specify date of antibiotic: DD / MM / YYYY \_\_\_\_\_

Oral Cholera vaccine (OCV) received: ☐ Unknown ☐ No ☐ Yes, When? DD / MM / YYYY \_\_\_\_\_

Name of OCV: \_\_\_\_\_

Relevant travel history: \_\_\_\_\_

**Signs & symptoms**

☐ Watery stool \_\_\_\_\_ days ☐ Respiratory distress \_\_\_\_\_

☐ Nausea \_\_\_\_\_ days ☐ Severe thirst \_\_\_\_\_

☐ Vomiting \_\_\_\_\_ days ☐ Unable to drink or drink poorly \_\_\_\_\_

☐ Fever \_\_\_\_\_ days ☐ Skin pin-pointing back very slowly (>2 seconds) \_\_\_\_\_

☐ Swelling \_\_\_\_\_

☐ Loss of consciousness \_\_\_\_\_

☐ Rapid or weak pulse \_\_\_\_\_

Other symptoms: \_\_\_\_\_

Any known contacts with anyone with similar symptoms? ☐ No ☐ Yes, specify: \_\_\_\_\_

**To be completed upon reception of the specimen by the receiving laboratory** \_\_\_\_\_

Receiving laboratory (name address or stamp) \_\_\_\_\_

Name of laboratory personnel receiving the specimen: \_\_\_\_\_

Date and time of specimen received: DD / MM / YYYY Hour / Minute \_\_\_\_\_

Condition of specimen (packaging, documentation): ☐ Adequate ☐ Not adequate

How adequately, specify (e.g. missing missing information, inadequate transportation or conservation temperature)? \_\_\_\_\_

Follow-up actions: ☐ obtain a second sample ☐ complete missing information ☐ other, specify: \_\_\_\_\_

2. A line must be drawn across the top of the form and the name of the health facility must be written in the space provided.

Page 2 of 2 (5/2013) GTFCC Form

<https://www.gtfcc.org/resources/gtfcc-laboratory-referral-and-results-reporting-forms/>

# Completing the RDT results for samples referred to the laboratory

2

1 — Was an RDT performed on the same specimen? ☐ No ☐ Yes, specify: ☐ Enriched RDT ☐ Direct RDT

3 — **Result:** ☐ Reactive O1 ☐ Reactive O139 ☐ Reactive O1 and O139 ☐ Non-reactive ☐ Invalid

4 — Name of RDT kit used: .....

1

**Yes** = RDT performed on same sample as sent to the laboratory

**No** = RDT not performed

2

**Direct** = RDT done using fresh stool

**Enriched** = RDT done using stool incubated in APW for 6 - 8 hours

3

Report the RDT result, which lines were reactive

4

RDT manufacturer and kit name

# Examples

1

Was an RDT performed on the same specimen? ☒ No ☐ Yes, specify: ☐ Enriched RDT ☐ Direct RDT

**Result:** ☐ Reactive O1 ☐ Reactive O139 ☐ Reactive O1 and O139 ☐ Non-reactive ☐ Invalid

Name of RDT kit used: .....

\_\_\_\_\_

No RDT  
performed

2

Was an RDT performed on the same specimen? ☐ No ☐ Yes, specify: ☐ Enriched RDT ☐ Direct RDT

**Result:** ☐ Reactive O1 ☐ Reactive O139 ☐ Reactive O1 and O139 ☐ Non-reactive ☐ Invalid

Name of RDT kit used: .....

\_\_\_\_\_

No information, lab  
is confused - was  
test not performed  
or not reported?

3

Was an RDT performed on the same specimen? ☐ No ☒ Yes, specify: ☐ Enriched RDT ☒ Direct RDT

**Result:** ☒ Reactive O1 ☐ Reactive O139 ☐ Reactive O1 and O139 ☐ Non-reactive ☐ Invalid

Name of RDT kit used: ..... *Bioline VC O1 /O139* .....


\_\_\_\_\_

Yes RDT performed  
on fresh stool,  
reactive O1 result  
using Bioline VC  
O1/O139 test






# What to do now



If there is no currently known outbreak or the outbreak has not been confirmed yet, send any RDT+ sample immediately to a laboratory for further confirmation.



If an outbreak has been confirmed, send at least 3 RDT+ samples per week per surveillance unit for further lab confirmation.

For more information [Public Health Surveillance for Cholera.](#)



# Links to GTFCC support material

Recommendations for public health surveillance for cholera :

<https://www.gtfcc.org/resources/public-health-surveillance-for-cholera/>

Rapid Diagnostic Test (RDT) for cholera detection :

<https://www.gtfcc.org/resources/rapid-diagnostic-test-rdt-for-cholera-detection/>

Quick reference guide for reading of cholera Rapid Diagnostic Tests (RDT) :

<https://www.gtfcc.org/resources/rapid-diagnostic-test-rdt-for-cholera-detection/>

Laboratory referral form for cholera suspected case :

<https://www.gtfcc.org/resources/gtfcc-laboratory-referral-and-results-reporting-forms/>

Template cholera case report form :

<https://view.officeapps.live.com/op/view.aspx?src=https%3A%2F%2Fwww.gtfcc.org%2Fwp-content%2Fuploads%2F2024%2F03%2Fgtfcc-template-cholera-case-report-form.docx&wdOrigin=BROWSELINK>

# END OF MODULE ASSESSMENT



# Assessment

1. RDTs should ideally be performed on fresh stool specimens within 4 hours of collection from a patient who has been ill for less than 2 days and who has not been given antibiotics.

True or False

2. If the RDT indicates the presence of O1, and the control line is not visible, then the result is considered to be reactive.

True or False

3. It is important to report non-reactive test results and whether or not an RDT has been performed.

True or False

4. Cholera RDTs cannot be used for confirmation of a case of cholera.

True or False

# Assessment

5. A rectal swab can be used for an RDT.

True or False

6. If the RDT shows bands for O1 and O139, and the control band is visible, what would you do? (select all appropriate answers)

Redo test

Report as O1 and O139 reactive

Report as invalid

Send a sample

7. How would you report a RDT test where no lines are seen?

Was an RDT performed on the same specimen? ☐ No ☒ Yes, specify: ☐ Enriched RDT ☒ Direct RDT

**Result:** ☐ Reactive O1 ☐ Reactive O139 ☐ Reactive O1 and O139 ☐ Non-reactive ☐ Invalid

Name of RDT kit used: *Bioline VL 01 / 0139* .....

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# Assessment Answers

1. RDTs should ideally be performed on fresh stool specimens within **4 hours** of collection from a patient who has been ill for less than **2 days** and who has not been given antibiotics.

**False** *to refresh your memory go to slide 11*

2. If the RDT indicates the presence of O1, and the control line is not visible, then the result is considered to be reactive.

**False the result would be invalid without the control line** *to refresh your memory go to slide 33 and 36*

3. It is important to report negative test results and whether or not an RDT has been performed.

**True, this avoids unnecessary repeat testing and provides vital epi information** *to refresh your memory go to slide 43*

4. Cholera RDTs cannot be used for confirmation of a case of cholera.

**True** *go to refresh your memory go to slide 12*

# Assessment Answers

5. A rectal swab can be used for an RDT.

False or only with additional step go to slide 11 to refresh your memory


6. If the RDT shows bands for O1 and O139, and the control band is visible, what would you do? (select all appropriate answers)

Redo test and report the second results, if RDT results remains O1 and O139 send sample for lab testing go to slide 40 to refresh your memory

7. How would you report an RDT test where no lines are seen?

Was an RDT performed on the same specimen? ☐ No ☒ Yes, specify: ☐ Enriched RDT ☒ Direct RDT

**Result:** ☐ Reactive O1 ☐ Reactive O139 ☐ Reactive O1 and O139 ☐ Non-reactive ☐ Invalid

Name of RDT kit used: Bioline VC 01 /0139 

Go to slide 47/48 to refresh your memory