MODULE 3 SCRIPT: GTFCC recommendations. CHOLERA RAPID DIAGNOSTIC TESTS (RDTs). *V1.0 November 2024.*

Slide 1: GTFCC recommendations. CHOLERA RAPID DIAGNOSTIC TESTS (RDTs).

Welcome to the third module of this course in which will discuss cholera Rapid Diagnostic Tests, or RDTs.

Slide 2: Learning objectives.

At the end of this module, you will be able to describe RDTs, explain when and why use RDTs. You will understand their limitations and know the procedure to perform RDTs for cholera. You will also be able to interpret RDT results and troubleshoot both the procedure and the results.

Slide 3: Learning outline.

This module is divided into the following 5 sections.

Slide 4: The result of any laboratory examination is only as good as the sample received in the laboratory.

Before we begin, remember the result of any laboratory examination is only as good as the sample that was received by the laboratory. Refer back to the previous module to ensure the best quality samples to perform RDTs on.

Slide 5: RDTS – WHAT • WHY • WHEN.

Let's dive into our first section. Rapid diagnostic tests, what are they, why do we use them and when do we use them

Slide 6: What are RDTS?

Rapid diagnostic tests or RDTs are lateral flow immuno-chromatographic assays. The RDTs commonly used for cholera testing detect up to two “types” or serogroups of *Vibrio cholerae* through detection of the O1 antigen or both O1 and O139 antigens.

They work on stool samples and do not require any complex laboratory set up.

The tests present themselves in two formats represented on the images on the right. They can be in a dipstick format or a cassette format. Just like COVID tests or home pregnancy tests except that they are performed on stool.

Slide 7: Products available.

There are a variety of commercially available RDT. In fact there are at least 22 brands currently on the market. Each have different levels of accuracy or performance making many of them less appropriate for use and none of them are WHO pre- qualified as of yet. However, the WHO recommends and distributes two types: the Crystal VC O1/O139 and the SD Bioline O1/O139. The crystal VC is a dipstick while the SD Bioline is a cassette.

Slide 8: Why use RDTs?

Slide 9: Why use RDTs?

Slide 10: Summary.

We described the GTFCC strategy in module 1 of this course, remember this was the summary table from Module 1 where you can see it is recommended.

To summarize:

When there is no outbreak we aim to quickly detect the first case to quickly protect the community and alert other departments to contain the disease and prevent widespread outbreaks. To do this, if RDTs are available, we test all suspected cases using the RDT’s. We also send the RDT reactive samples to the laboratory for confirmation by Culture or PCR. If RDTs are not available, we simply collect samples from all suspected cholera cases and send them to the laboratory for testing as soon as possible. We record all RDT results (reactive or non reactive) and report any reactive RDT result to the health authorities on the same day.

When there is an outbreak of cholera in the area or surveillance unit, we aim to monitor the outbreak. If RDTs are available, we test the first 3 suspected cases of the day in our health facility using RDTs. We also send the reactive RDT sample to the laboratory for confirmation by culture or PCR. If RDT are not available for any reason, we collect samples from the first 3 suspected cases of the week in the health facility and send them to a laboratory for testing. We keep record of all RDT results. Finally, we report all suspect cases and RDT results to health authorities on a weekly basis.

Slide 11: Samples for RDTs.

Slide 12: RDTs - Limitations.

RDT also have limitations. They cannot be used to confirm *Vibrio cholerae* O1 or O139 and therefor never replace culture or molecular confirmation. They cannot confirm the diagnosis of a case of cholera.

Patient treatment should never depend on the RDT result. Treatment must be based on the level of dehydration of the patient.

The RDTs that are available today do not detect the cholera toxin.

And finally, RDTs do not provide us with any antimicrobial susceptibility data.

Slide 13: Safety.

A few safety notes before we discuss the procedure.

Slide 14: Safety first.

Slide 15: PROCEDURE.

Let’s look at the procedure for a Rapid Diagnostic Test.

Slide 16: Considerations prior to testing.

Before you start, always return to the manufacturer’s specifications. Look at the procedure that is provided with the kit that you are using.

Slide 17: Supplies and material.

Here is the minimum list of supplies and materials you will need to perform RDT tests.

Gloves and scrubs/labcoat – to protect yourself and avoid contamination Hazardous waste bin or bag to dispose of materials after use

Tissue paper – for cleaning surfaces and materials and incase of any spills which might occur

Test kit – this contains materials needed for testing, like droppers or tubes as well as the actual test.

Timer – to ensure the correct time for reading the test is followed, you can also use the timer on your phone if necessary. Be careful to avoid contaminating it!

Register/forms – The correct paperwork to document the test was performed and the results. All tests should be documented even if invalid or negative

Marker/pen – To write on the test and to fill in the paperwork

Slide 18: Crystal® VC O1/O139, Arkray Dipstick method.

Note to presenter - Only review this section is country orders and uses Arkray tests to avoid confusion.

Crystal® VC O1/O139, Arkray, the Arkray RDT test is a strip with test reagents on it we call this a dipstick test. It is one of the 2 GTFCC recommended test kits available to test for *Vibrio Cholerae*. Here we will look at the standard operating procedurers or SOP needed to perform the test correctly to ensure accurate reliable results.

Slide 19

After putting on your gloves and preparing all the material required, start by labeling the sample processing vial and test tube with the correct patient ID as it is written on the sample collection container.

Open the cap of the sample processing vial provided in the kit and the sample container. Depending on the condition of the stool – semi-solid or liquid select the best sample collection method. You don’t need a lot of stool in the processing vial for the test.

If the sample is semi solid, use the collection stick attached to the vial to transfer a small amount of stool into the bottle and close the lids of the vial and the stool cup. "Stab" do not "scoop" the stool to avoid picking up particulate matter that may clog the dipstick membrane.

If the sample is liquid, use a plastic pipette to transfer 2 drops of the liquid into the vial. Close the lids of the vial and the stool cup and discard the dropper into the waste container.

Slide 20

Check the lid is tightly fastened before shaking the contents to mix. You can use a tissue to cover the lid as an extra precaution to avoid leaks and spills while shaking.

Remove the transparent cap then the tip of the vial can be snapped off. Always make sure to point the tip away from you and use the tissue to catch any splashes.

Dispense 4 drops of the stool/buffer mixture into the labeled tube.

Discard the used sample vials in the biowaste container.

Slide 21

Carefully open the test kit. If it is damaged, or the desiccant is missing or has changed color, throw it away and use another test.

Only touch and hold the dipstick from the top end.

Write the patient’s name or ID on the dipstick as indicated on these pictures and set the timer to 15 mins.

Place the labelled dipstick arrow end down into the sample mix and start the timer. The end of the dipstick must be submerged in the sample but not the arrows.

Set up any other tests and wait the 15 mins.

Dispose of the test dipstick after reading in the biowaste container.

Slide 22: Bioline™ Cholera Ag O1/O139, Abbott Cassette method.

Note to presenter - Only review this section if country orders and uses Bioline tests to avoid confusion.

Bioline™ Cholera Ag O1/O139, Abbott RDT test is contained in a plastic case known as a cassette, It is one of the 2 GTFCC recommended test kits available to test for *Vibrio Cholerae*. Here we will look at the standard operating procedures or SOP needed to perform the test correctly to ensure accurate reliable results.

Slide 23

After putting on your gloves and preparing all the material required, start by labeling the specimen collection tube with the correct patient ID as it is written on the Specimen collection tube

Open the cap of the sample processing vial provided in the kit. Take care to unscrew the entire lid and not the small cap!

Depending on the condition of the stool – semi-solid or liquid select the best sample collection method. You don’t need a lot of stool in the Specimen collection tube for the test.

If sample is semi solid, use a swab to transfer a small amount of stool into the bottle, swirl the swab at least 10 times to transfer the sample into the liquid.

If the sample is liquid, use the provided plastic pipette. The pipette has a line which shows you how much sample to collect. Transfer the liquid in the pipette into the vial. Note – do not use other pipettes as the fill line may not be the correct volume for this test.

Slide 24

Take care when removing the swab, gently squeeze the swab against the side of the container so the liquid remains in the container.

Close the lids of the vial and any sample containers used. Discard the swab or dropper into the biohazard waste container.

Slide 25

Check the lid is tightly fastened before shaking the contents to mix. You can use a tissue to cover the lid as an extra precaution to avoid leaks and spills while shaking.

Twist open the small cap of the specimen collection lid.

Slide 26

Carefully open the test pouch. If it is damaged, or the desiccant is missing or has changed color, throw it away and use another test.

Write the patient’s name or ID on the cassette as indicated on these pictures and set the timer to 15 mins.

Holding the sample tube straight, gently dispense 3 drops into the specimen well – marked with an S. Be careful to add 3 drops, adding too much or too few drops can change the test results.

Start the timer and discard the specimen collection tube. Set up any other tests and wait the 15 mins.

The result must be read after 15 mins, waiting longer than this can lead to false results being reported.

Dispose of the test cassette after reading in the biowaste.

Slide 27: Troubleshooting the procedure.

Pay attention to these common causes of error during the procedure that can cause issues with the results.

* The RDT may not be stored as the manufacturers recommend or they may be expired
* Manufacturers may have changed the procedure and the user hasn’t checked the new instructions
* You may have added too much or too little sample to the specimen buffer or to the test cassette
* An item in the kit may be damaged

Slide 28: READING THE RESULTS.

Let’s look at the results and the interpretation of the results.

Slide 29: Waiting to read the results.

After adding the sample to the test, you must wait to read the results. The amount of time you wait depends on the test kit so please refer to the kit instructions for specifics.

For Arkray dipstick, it is recommended to wait 15 to 30 minutes then to remove the dipstick from the test tube and read the results.

For Bioline cassettes, it is recommended to wait 15 minutes exactly and read the results.

It is important to stick to the recommended time. Waiting too little may lead to false non-reactive (negative) readings while waiting too long could lead to false reactive (positive) readings or false negatives as bands can change over time.

Slide 30: Results: Reactive / non-reactive.

When reporting RDT results we use the term – Reactive or Non-reactive instead of Positive or Negative this is to communicate the results in a more accurate way. RDT’s do not confirm Cholera, so we avoid saying positive as it can give the impression that a case has been confirmed. We use reactive to indicate that a test line has had a reaction with the tested sample.

We report reactive for each test line which is seen on the test rather than just saying “cholera positive” which doesn’t tell us much information. In this this way we accurately communicate the added information of which serogroup reacted.

On the other hand, since RDT’s have limitations, we report Non-reactive instead of negative as negative implies a certainty which the test doesn’t provide.

Slide 31: Results: Dipstick.

Note to presenter – You can review test results for tests in use to avoid confusion.

For Akray dipsticks, the results could look like this with presence or absence of control bands, or bands for VC O1 or VC O139.

Slide 32: Results: Cassette.

Note to presenter – You can review test results for tests in use to avoid confusion.

For Bioline cassettes, these are the different possible results with the presence or absence of control lines, or lines for VC O1 or VC O139.

Slide 33: Key recommendations for reading of results.

Key recommendations to always remember:

RDT test kits, even coming from the same manufacturer, may have different positions for test and control bands/lines on the test. Please refer to the instructions provided with the specific RDT in use for correct reading instructions.

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.

Slide 34: Control band.

The first thing to do when reading the results, is to check to see if there is a control band. If this band or line does not appear, the test is invalid and you must not report results.

All 4 Arkray dipsticks on the right of this image, are invalid because of the absence of a control band.

Slide 35: Control band.

For Bioline cassettes, these are the different possible results. The first thing to look for is the presence or absence of control lines, then lines for VC O1 or VC O139.

All 4 cassettes on the right of this image, are invalid because of the absence of a control band.

Slide 36: Invalid: reading.

When no control band is seen, regardless of any other lines, you must record the initial invalid result. Repeat the test using a new test device. Report the final result. If the result remains invalid it may be appropriate to send the sample for further testing at the laboratory.

If results from many samples are invalid refer to troubleshooting methods. A high number of invalids can indicate a problem with testing methods or the kits.

Slide 37: O1 and O139 non-reactive: reading.

When the control band is apparent, but there are no bands for O1 or O139 the result can be interpreted as non-reactive (negative). You can then report the RDT as a Non- reactive result.

Slide 38: O1 reactive: reading.

When the control and O1 bands are observed, and the O139 band is not observed, this is a reactive RDT result for O1. You must report this as RDT reactive for O1.

Slide 39: O139 reactive: reading.

Now, if both control and O139 bands are observed but not the O1 band, let us pause a little. The test is valid because there is a control band and it appears to be O139 reactive.

However it is good to know that *Vibrio cholerae O139* are not yet found outside of certain regions of South Asia. So the result should be considered in your country context.

The O139 test lines do not always perform well and sometimes we have false results, where a band is seen for O139. If this is an unexpected result in your context we recommend to redo the test, and report that result.

If you obtain the same result, O139 reactive, send the initial sample to a laboratory for further confirmation.

Slide 40: O1 and O139 O139 reactive: reading.

This is a similar situation where all control, O1 and O139 bands are observed. Let us pause again. The test is valid because there is a control band and it appears to be both O1 and O139 reactive.

This is however an unusual result. Keeping in mind what we said about O139 being rare and mostly found in South Asia, it is even more rare to find a patient that has been infected with both VC O1 and O139. In fact this has never been documented.

So this may be false or incorrect line, for O139 while still being a correct reaction, Reactive, for O1. Again we recommend redoing the test, and reporting that second result. If you obtain the same result, report and send the initial sample to a laboratory for further confirmation.

When you report on these types of results, a comment can be added to the report.

Slide 41: Troubleshooting the results.

Difficulties with the reading test results may arise in certain situations. These could be linked to:

* Not waiting the appropriate amount of time before reading the results (waiting too long or too little)
* The Misreading of results (for example when we fail to check which band corresponds to which result)
* The absence of a control band which directly invalidates the test
* And finally, incorrect results can rarely occur either showing a band where there shouldn't be a band, or not showing a band where there should be a band.

Think if it this way, when you have a reactive RDT for cholera VC O1, there is a strong probability that the patient has cholera but you do not have 100% certainty.

When you have a non-reactive RDT, there is even a stronger probability that the patient does not have cholera but you are not 100% certain.

Treatment and care should be provided to a suspected case on the basis of their symptoms and level of dehydration, and not on the basis of an RDT result.

Slide 42: REPORTING.

Reporting.

Slide 43

All RDT results should be recorded immediately, as soon as an RDT is read, into a local log or register. You should record the results even if the test is invalid or needs to be repeated.

All RDT results should also be reported in the appropriate surveillance and laboratory referral forms to the health authorities, again even if they are invalid or non reactive.

It is important to account for all tests used, so 20 tests in a kit will have 20 results in the logbook for accurate stock forecasting.

This can also be critical to identify potential challenges with the test. Too many invalids or false results may indicate that staff need a refresher training, or that the kit is not working as expected. This can be communicated up the chain of command to resolve the issue and avoid wasting time, effort and resources.

Slide 44: Reporting RDT results.

RDT results should be reported into the national cholera surveillance database through the surveillance teams so that an outbreak can be appropriately responded to or to adapt a response to an outbreak. In this case there is generally a reporting mechanism in place that can involve reporting the RDT results in the case investigation forms.

If a sample is being sent to the laboratory and an RDT was performed in the field, then it is good practice to share the RDT result with the laboratory too. They will take into account these results when they further analyze the sample. In this situation, patient and sample information together with the RDT result are shared through the sample referral form.

Slide 45: Key recommendations for reporting.

When reporting the RDT result, pay particular attention to using the correct patient or sample ID.

As always, saying when you have no information is information by itself. For example if an RDT was not performed it is best to report “RDT not done” than to leave a space empty.

And finally, before reporting an O139 reactive result, “pause and reflect”. Did you redo the test first? Does this result make sense?

Slide 46: GTFCC Laboratory referral form for cholera suspected case.

Slide 47: Completing the RDT results for samples referred to the laboratory.

[Note for script: they will have to do this on the case report form as well]

Let’s look at how to correctly complete the example GTFCC laboratory referral form for clear communication. This is the level of information which is recommended for completion. There are only 4 things to fill in but this small amount of information can tell the laboratory and Epi teams a lot and is important to ensure the laboratory can follow the testing strategy and provide the best quality information to inform the response.

1. Was the RDT performed on the same specimen as is sent to the laboratory, this can be used by the laboratory to compare the laboratory results to the RDT, if the samples were not the same it may explain why the results on the sent sample are different from the ones in the laboratory.

"Same specimen" means the patient sample was collected into the appropriate container, and either the sample was tested by RDT and then processed for transport, or one pot was collected to be tested by RDT and a second portion of sample was processed for transport. In both cases the sample was from the same patient from the same collection time.

A sample from the same patient collected at a different time is not the same sample.

2. It is rare for the healthcare facility to perform RDT’s on “enriched” samples. To do this requires additional reagents and equipment as well as an 8 hour incubation time. It is most common and encouraged to perform the RDT directly from the stool sample. Tick the method your facility used.

3. There are 4 options for results – the control and VC O1 line reacted, – the control and VC O139 line reacted, –the control and VC O1 and O139 line reacted or the control line was absent. Always remember to check the control line is visible before reading the results.

4. Write in the name and manufacturer of the test, this tells the laboratory if a recommended test kit is used, which results to expect, for example a facility using a test which only looks for VC O1 should never report reactive 0139. The lab and surveillance can also gather information on the test performance to ensure the test is working as expected.

Slide 48: Examples.

Lets look at some examples when completing the form:

1 – No RDT was performed, if this is a facility which should have RDT’s then the laboratory might be alerted to a stock out and that this facility requires more tests, or this may tell the Epi team this suspect case is from an area outside of the expected cholera risk area so a positive could modify the local response. Or this could be from a laboratory which doesn’t have RDT’s, depending on the epidemiological situation, the lab can determine if they should process this sample – if the site is in an outbreak and are sending 50 samples per week, this is outside the testing strategy, to save resources the laboratory would not test and may retrain the site on the correct testing strategy. You can see already how just this small piece of information can be used for action.

2 – This is the worst case scenario for the laboratory and epi teams. There is no information, none of the above decisions can be made. This can lead to tests being performed unnecessarily, waste or resources and time. If a test was performed and not recorded that test was essentially wasted, the results do not inform the response which can delay action.

3 – This is good information, The lab now knows that the RDT test was performed on the sample. The HCW did the test directly on the stool sample (without enrichment) and the result was reactive for VC O1 using one of the 2 GTFCC recommended kits.

Now when the laboratory finished testing they can compare their results to the RDT results and understand if there is a difference. Depending on the Epidemiological situation this clear information may alert the response teams to take action.

Clear data communication can mean the difference between a timely response and an outbreak.

Slide 49: What to do now.

You have performed a rapid diagnostic test and you have a RDT reactive result. You have reported that result. What should be done now?

We refer you back to the testing strategy in place in your country.

Ideally, if there is no confirmed cholera outbreak in your area the sample that is RDT reactive should be sent to the laboratory of reference for them to confirm cholera. If there is an ongoing confirmed outbreak, there is no longer a need to laboratory- confirm all RDT reactive results. The GTFCC recommends in that case, only 3 RDT reactive samples per week per surveillance unit be sent to a laboratory for confirmation and further characterization.

Slide 50: Links to GTFCC support material.

Further information you can access to support your learning.

Slide 51-53: END OF MODULE ASSESSMENT.