

Troubleshooting guide

For supervisors overseeing users of malaria RDTs

Developed by FIND in collaboration with the John's Hopkins Bloomberg School of Public Health (JHSPH), Malaria Consortium (MC), Population Services International (PSI), and the Global Malaria Programme of the World Health Organization (WHO-GMP), with funding from UNITAID. Some drawings are based on generic materials available at <http://www.finddx.org/implementation-tools/#malaria>



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Foreword

This guide provides practical recommendations for solving problems that may arise when using malaria rapid diagnostic tests (RDTs), and gives simple instructions on what actions to take if problems persist. It is designed to be a complementary tool to training and supervision, for use by supervisors overseeing the work of RDT users in any health care setting where RDTs are used. Although the list of problems has been selected based on extensive experience from various field studies as well as the RDT Lot Testing Programme, it is not exhaustive.

Any suggestions for improvement or reports of problems not covered in this guide should please be sent to info@finddx.org.

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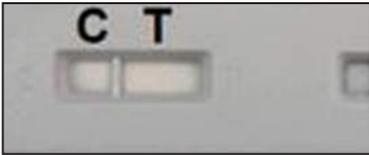
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Section 1

Troubleshooting unusual RDT results

Invalid tests



Control line is absent;
test line is also absent.



Invalid RDT result



Another RDT should be opened
to repeat the test. If the problem
persists, follow instructions in
section 4.



Control line is absent;
test line is present and
visible.

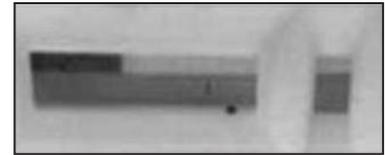


Invalid RDT result



Another RDT should be opened
to repeat the test. If the problem
persists, follow instructions in
section 4.

Structural problems



RDT strip is misplaced or shifted,
e.g. shifted to the right, left, or up so
that it cannot be seen in the sample
well

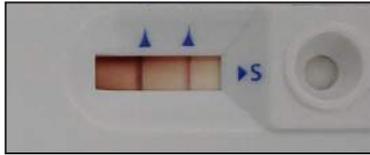


RDT cannot be used



Another RDT should be opened
to repeat the test. If the problem
persists, follow instructions in
section 4.

Red background



Light background only.

Both the test and control lines are present and visible.



Positive RDT result

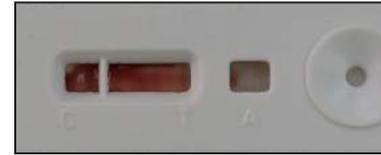


Light background only.

Control line is present.
Test line is absent, but not hidden (obscured) by the red background.



Negative RDT result



Strong red background.

Control line is present and visible, but test line is hidden by the red background.



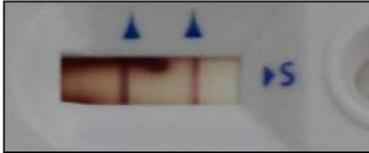
Cannot be sure about the test result



Another RDT should be opened

to repeat the test. If the problem persists, follow instructions in section 4.

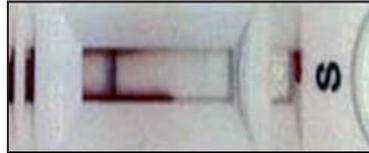
Incomplete clearing (red staining)



Red staining does not obscure the test and control lines. Both **control line and test line are present and visible**.



Positive RDT result



Red staining not obscuring Test and Control lines. Control line is visible but **test line is absent**.



Negative RDT result



Red staining obscures the area where the test line should be. Control line is visible, but it is **unclear whether the test line is present or absent**.



Cannot be sure of the test result



Another RDT should be opened to repeat the test. If the problem persists, follow instructions in section 4.

Failure to flow



Blood and buffer **do not run the length of the strip**. Test line is present and visible but there is **no control line**.



Invalid RDT result



Another RDT should be opened to repeat the test. If the problem persists, follow instructions in section 4.



Blood and buffer **do not run the length of the strip**. There is **no control line** and **no test line**.

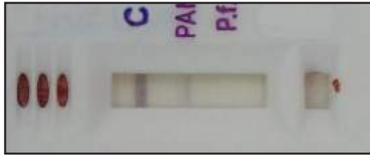


Invalid RDT result



Another RDT should be opened to repeat the test. If the problem persists, follow instructions in section 4.

Diffuse test line

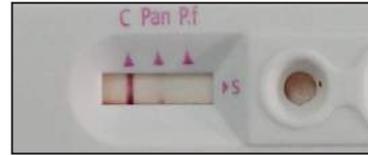


Test line is wider than the control line, **with blurred edges**.



Visible test lines should be read as positive, even if they are wider than the control line or have blurred edges => Positive RDT result.

Patchy broken test line



Test line is visible but interrupted (**broken**).



Visible test lines should be read as positive, even if they appear incomplete => Positive RDT result.



Possible problems

Possible causes

- For a **red background** or **incomplete clearing**

- too much blood
- not enough buffer
- reading time too short

- For **failure to flow**

- not enough buffer
- blood is collected too slowly (blood clotting) or contains residuals of cotton wool

- For suspected **false negative** results

- not enough blood
- too much buffer
- reading time too short
- *Pfhrp2* gene deleted parasites

A few tips to solve problems (1/4)

■ Correct RDT procedure

- ▶ Check again the job aid instructions:
 - Blood volume?
 - Buffer volume?
 - Reading time?

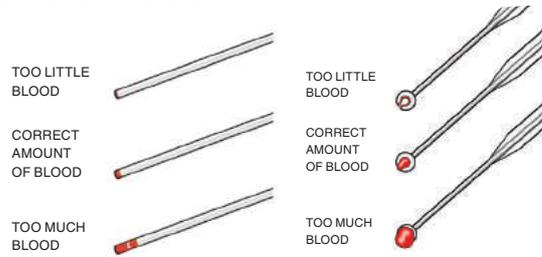


■ Correct blood collection and volume

- ▶ Clean the finger well, ensuring there are no residuals of alcohol or cotton wool.



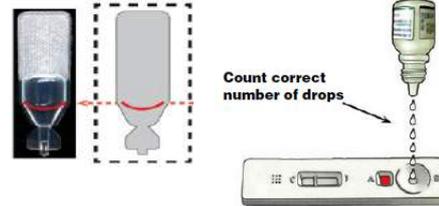
- ▶ Correct use of the transfer device:



A few tips to solve problems (2/4)

■ Correct buffer volume

- ▶ For vials, buffer should be at or above the level of the red arrow; for bottles, count the correct number of drops as per manufacturer's specifications.

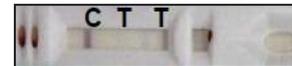


■ Correct reading time and interpretation of test lines

- ▶ Wait for the correct amount of time before reading the test results – between 15-20 minutes depending on the manufacturer's specifications.

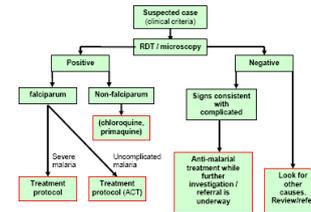


- ▶ Even faint test lines should be read as positive test results.



A few tips to solve problems (3/4)

- Correct interpretation of symptoms
- ▶ Look closely at the case management algorithm;¹ interpret test results in view of the patient's history and symptoms.



¹ This flowchart is only for illustration, national guidelines for case management should be followed.

- Good quality of microscopy (if done for same patient)
- ▶ If microscopy was done for the same patient, verify its quality (microscope, competency, reliability, etc.) and interpret differences in test results in view of the context.



A few tips to solve problems (4/4)

- Suspected *pfrp2* gene deletion
 - ▶ Perform a second quality assured HRP2-based RDT.
 - ▶ If the second RDT is negative, perform the testing with a pan or pf-pLDH-based test (if available) and perform quality microscopy.
 - ▶ If the pan and/or pf-pLDH test lines are positive and microscopy is positive by two qualified microscopists, prepare dried filter paper spots for PCR confirmation of *P. falciparum* and *pfrp2/3* gene analysis.

Section 2

Troubleshooting issues with kit accessories



Accessories: instructions for use, problems and recommendations (1/2)

■ Alcohol swab



▶ Open the alcohol swab pack just before use. Wipe the patient's finger and let it air-dry. Don't blow on the finger, and don't use a cloth or piece of paper to dry it, as this could re-contaminate the finger.

If the alcohol swab is dry:

Use another alcohol swab, or use cotton wool with 70% alcohol. If this happens repeatedly, alert your supervisor or focal contact (details in section 4).

■ Lancet



Fig. 1

▶ Open the lancet pack just before use. Prick the patient's finger and wipe away the first drop of blood. Allow the finger to dry completely (alcohol may interfere with testing). Discard the lancet in the sharps box **immediately** after pricking.

If the blood drop is too small:

Ask the patient to rub his/her hands to stimulate blood circulation. Gently squeeze the finger by pushing towards the fingertip to produce a larger drop. Prick with a quick firm stab (a slight prick will only produce small drops) on the side of the finger (as shown in Fig. 1). Gently squeeze the finger to produce a big drop of blood.

Accessories: instructions for use, problems and recommendations (2/2)

■ Buffer bottle



- ▶ The volume in the bottle should be enough for all tests in the box. The colour should be consistent (if it can be seen, e.g. transparent bottle).

■ Buffer vial



- ▶ Before opening the vial, tap it to ensure all buffer has settled. The vial should have enough buffer volume for one test. The colour and volumes should be consistent for all vials.

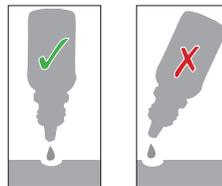
■ For both bottles and vials

- ▶ Hold the bottle/vial vertically, squeezing it gently and slowly to get free falling drops. Count the exact number of drops as recommended for testing.

If there is insufficient volume, or the bottles or vials are empty, or the buffer colour is unusual

(i.e., different from other bottles or vials in the same RDT lot):

Use another bottle or vial from the same RDT lot. Don't use bottles or vials from another lot (even if it is the same brand and product), or from another RDT product (even if it is an RDT for malaria or an RDT from the same manufacturer). Never use water! In any case: Follow instructions in section 4.





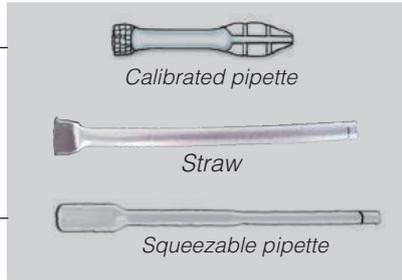
Section 3

Troubleshooting issues with blood transfer devices

Blood transfer devices: overview of how to use

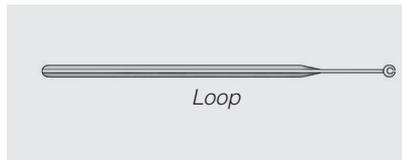
(Detailed instructions and recommendations on pp 19–28)

Gently squeeze the device before touching the blood drop, and then draw the blood up to the (first) mark by gently and slowly releasing the pressure.



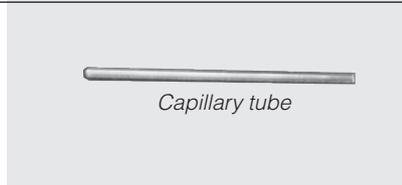
During the blood collection: don't release the pressure too suddenly and don't lift the device as this may allow air bubbles to enter.

Touch the blood drop with the tip of the loop and let the loop fill completely with blood.



To fill the loop, ensure a good sized blood drop and good contact with the gap at the bottom end of the loop.

Hold the capillary tube vertically, placing the tip of the capillary tube on the blood drop and drawing the blood up to the mark.



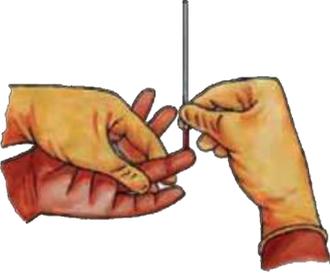
Don't move the device and don't lift it while the blood is being drawn. Lift it as soon as the blood reaches the mark.

Apply the inverted cup gently to the top of the blood drop and let the cup fill with the blood.



Don't press the cup against the finger but just gently touch it to the blood drop. Let the cup fill without moving or lifting it.

Capillary tube (1/2)

| | |
|-------------------------|--|
| Fingerprick | <ul style="list-style-type: none">▶ To obtain a nice big drop before pricking, massage the finger to stimulate blood circulation.▶ Prick the patient's finger with a quick firm stab. Gently squeeze the finger to produce enough blood. |
| Blood collection |  <ul style="list-style-type: none">▶ Ensure a good-sized drop is on the finger before collecting.▶ Place the tip of the capillary tube on the top of the blood drop, by holding it in vertical position. Let the blood go up automatically, and then lift the tube when the blood reaches the mark. <p><i>Note:</i> Do not collect blood twice using the same blood transfer device if the collection fails. You must use a new one.</p> |
| Transfer | <ul style="list-style-type: none">▶ During the transfer of blood from the finger to the RDT, don't make any sudden movements or touch anything with the transfer device (either could lead to spillage of blood from the device). |

Capillary tube (2/2)

| | | |
|----------------------|---|--|
| Blood deposit |  | ➤ Add the blood to the RDT sample well by holding the capillary tube vertically. The tip of the tube needs to be in full contact with the RDT strip until the whole blood volume has been released onto the RDT pad. |
|----------------------|---|--|

Possible problems

- I have air bubbles in the device.
- It is difficult to fill the device with blood exactly to the mark.

Possible solutions

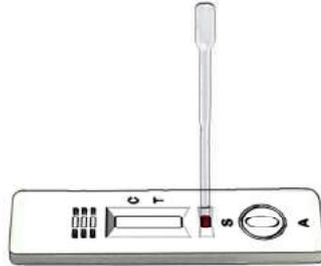
- As the blood is being drawn into the device, don't move or lift it, as this may allow air bubbles to enter.
- Keep the device in a vertical position and in contact with the blood, without pressing it against the finger. The blood will slowly be drawn up due to the capillary force.
- As soon as the blood reaches the mark, lift the device. This will avoid the blood going above the mark.

Pipette (1/2)

| | |
|-------------------------|--|
| Fingerprick | <ul style="list-style-type: none">➤ To obtain a nice big drop before pricking, massage the finger to stimulate blood circulation.➤ Prick the patient's finger with a quick firm stab. Gently squeeze the finger to produce enough blood. |
| Blood collection |  <ul style="list-style-type: none">➤ Ensure a good-sized drop is on the finger before collecting.➤ Gently squeeze the pipette bulb, and then gently apply its tip to the blood drop (don't press it against the finger). Gently and slowly release the pressure on the bulb to draw the blood up until it reaches the mark. Then, lift the pipette for transfer. <p><i>Note:</i> Do not collect blood twice with the same blood transfer device if the collection fails. You must use a new one.</p> |
| Transfer | <ul style="list-style-type: none">➤ During the transfer of blood from the finger to the RDT, don't make any sudden movements or touch anything with the transfer device (either could lead to spillage of blood from the device). |

Pipette (2/2)

Blood deposit



- ▶ Add the blood to the RDT sample well by holding the pipette vertically. The pipette tip needs to be in full contact with the RDT strip. Gently squeeze the pipette bulb until the whole blood volume has been released onto the RDT.

Possible problems

- I have air bubbles in the device.
- It is difficult to fill the device with blood exactly to the mark.
- The blood remains trapped in the pipette.

Possible solutions

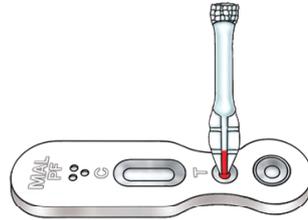
- As you draw the blood, keep the device in full contact with the blood and don't lift it, as this may allow air bubbles to enter.
- As soon as the blood reaches the mark, lift the device: this will avoid the blood going above the mark.
- Don't squeeze the pipette bulb too hard and don't release it too suddenly when drawing the blood, as this will create a strong aspiration of blood, causing blood to remain trapped in the pipette.

Squeezable pipette (1/2)

| | |
|-------------------------|--|
| Fingerprick | <ul style="list-style-type: none">▶ To obtain a nice big drop before pricking, massage the finger to stimulate blood circulation.▶ Prick the patient's finger with a quick firm stab. Gently squeeze the finger to produce enough blood. |
| Blood collection |  <ul style="list-style-type: none">▶ Ensure a good-sized drop is on the finger before collecting.▶ Gently squeeze the pipette bulb, and then lightly apply its tip to the blood drop (don't press it against the finger). Gently and slowly release the pressure on the bulb to draw blood until it reaches the first mark. Then lift the pipette for transfer. <p>Note: Do not collect blood twice with the same blood transfer device if the collection fails. You must use a new one.</p> |
| Transfer | <ul style="list-style-type: none">▶ During the transfer of blood from the finger to the RDT, don't make any sudden movements or touch anything with the transfer device (either could lead to spillage of blood from the device). |

Squeezable pipette (2/2)

Blood deposit



- Add the blood to the RDT sample well by holding the pipette vertically. The pipette tip needs to be in full contact with the RDT strip. Gently squeeze the pipette bulb until the whole blood volume has been released onto the RDT.

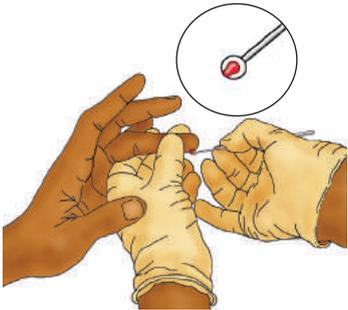
Possible problems

- I have air bubbles in the device.
- It is difficult to fill the device with blood exactly to the first mark.
- The blood remains trapped in the pipette.

Possible solutions

- As you draw the blood, keep the device in full contact with the blood and don't lift it, as this may allow air bubbles to enter.
- As soon as the blood reaches the first mark, lift the device. This will avoid the blood going above the mark.
- Don't squeeze the pipette bulb too hard and don't release it too suddenly when drawing the blood, as this will create a strong aspiration of blood, causing blood to remain trapped in the pipette.

Loop (1/2)

| | |
|-------------------------|--|
| Fingerprick | <ul style="list-style-type: none">▶ To obtain a nice big drop before pricking, massage the finger to stimulate blood circulation.▶ Prick the patient's finger with a quick firm stab. Gently squeeze the finger to produce enough blood. |
| Blood collection |  <ul style="list-style-type: none">▶ Ensure a good-sized drop is on the finger before collecting.▶ Hold the patient's finger with the blood drop facing downwards, and then touch the blood drop with the tip of the loop. Let the loop fill with blood. Lift the device only once the loop has completely filled with blood. <p>Note: Do not collect blood twice with the same blood transfer device if the collection fails. You must use a new one.</p> |
| Transfer | <ul style="list-style-type: none">▶ During the transfer of blood from the finger to the RDT, don't make any sudden movements or touch anything with the transfer device (either could lead to spillage of blood from the device). |

Loop (2/2)

| | | |
|----------------------|---|---|
| Blood deposit |  | <p>➤ Add the blood to the RDT sample well by holding the device at an angle as shown in the diagram. Gently press the loop against the strip. Maintain contact with the strip until the whole blood volume has been released.</p> |
|----------------------|---|---|

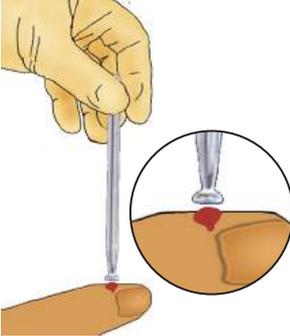
Possible problems

- It is difficult to fill the loop completely with blood.
- It is difficult to release the whole blood volume on the RDT.

Possible solutions

- For blood collection, ensure there is a big blood drop and a good contact between the drop and the open end of the loop.
- When drawing the blood, don't move the device and don't lift it until the loop has completely filled with blood.
- For the blood deposit, the open end at the tip of the loop needs to be in contact with the RDT sample pad. Don't move the device until all the blood has been absorbed by the RDT pad.

Inverted cup (1/2)

| | |
|-------------------------|---|
| Fingerprick | <ul style="list-style-type: none">➤ To obtain a nice big drop before pricking, massage the finger to stimulate blood circulation.➤ Prick the patient's finger with a quick firm stab. Gently squeeze the finger to produce enough blood. |
| Blood collection |  <ul style="list-style-type: none">➤ Ensure a good-sized drop is on the finger before collecting.➤ Place the tip of the inverted cup device on the blood drop, holding the device in a vertical position. The blood will be absorbed automatically. Only lift the device once the cup has filled with blood. <p>Note: Do not collect blood twice with the same blood transfer device if the collection fails. You must use a new one.</p> |
| Transfer | <ul style="list-style-type: none">➤ During the transfer of blood from the finger to the RDT, don't make any sudden movements or touch anything with the transfer device (either could lead to spillage of blood from the device). |

Inverted cup (2/2)

| | | |
|----------------------|---|--|
| Blood deposit |  | <p>➤ Add the blood to the RDT sample well by holding the inverted cup device vertically. The bottom end of the cup needs to be in full contact with the RDT strip. Wait until all the blood volume has been released onto the strip.</p> |
|----------------------|---|--|

Possible problems

- It is difficult to fill the cup completely with blood.
- It is difficult to release the whole blood volume onto the RDT.

Possible solutions

- For blood collection, ensure a big blood drop. The cup should be gently applied to the top of the blood drop and not pressed against the finger otherwise it cannot get filled with blood and the volume will not be correct.



- For blood deposit, hold the device vertically. If only one side of the cup touches the RDT pad, the blood will not be fully released.



Section 4

Instructions for acting on problems

If the problem occurs only in a few cases

'Few' means less than 10% of cases have a problem (e.g., less than three tests or accessories out of a box of 25)

- Supervisor should use the troubleshooting guide to identify and resolve the problem together with the RDT user
- Supervisor should conduct a refresher training, focusing on the sources of the problem, and emphasizing the critical steps of RDT testing
- Supervisor should reassure the RDT user that problems such as invalid tests, slight red background, etc. can occur at a low frequency

Note: Problems with buffer bottles/vials (e.g., bottles/vials being empty or having insufficient volume) should ALWAYS be reported to your point of contact (see next page).

If the problem persists, is critical or occurs frequently

'Frequently' means more than 10% of cases have a problem (e.g., three or more tests/accessories out of a box of 25)

Course of action

- Fill in the tally sheet and a reporting form if available
- Take pictures and collect samples of the problematic RDTs or accessories
- Report the problem to the diagnostics or QA/QC coordinator
- If instructed, conduct a survey to see if other RDT users have had similar problems

Whom to contact

Name:

Title:

Phone number:

Name:

Title:

Phone number:

Tally sheet for problems

| | Today's date | RDT provider/ID | RDT product name and catalog # | RDT lot # | Action taken |
|---|--------------|-----------------|--------------------------------|-----------|--------------|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5 | | | | | |
| 6 | | | | | |
| 7 | | | | | |

Problems reported to: _____ (name), on: _____ (date)

Tally sheet for problems

| | Today's date | RDT provider/ID | RDT product name and catalog # | RDT lot # | Action taken |
|---|--------------|-----------------|--------------------------------|-----------|--------------|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5 | | | | | |
| 6 | | | | | |
| 7 | | | | | |

Problems reported to: _____ (name), on: _____ (date)



Troubleshooting guide

For supervisors overseeing users of malaria RDTs



<http://www.finddx.org/implementation-tools/#malaria>