How to use a
G6PD Rapid Diagnostic Test

(for detecting glucose-6-phosphate dehydrogenase deficiency)

A guide for training at health facility level

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World Health Organization

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NOTE ON MODIFYING THIS MANUAL TO SUIT YOUR COUNTRY'S MALARIA CONTROL POLICIES

This manual and the accompanying material are designed to train health workers in the safe and accurate use of the glucose-6-phosphate dehydrogenase (G6PD) rapid diagnostic tests (RDT) following a diagnosis of *Plasmodium vivax* infection. The manual is targeted for a health facility level that has the capacity to safely administer and monitor a 2 week primaquine treatment regimen for radical cure of *P. vivax*. National guidelines for treatment of vivax malaria differ between countries and therefore will not be covered in this manual. Workers will also need separate training in case management for radical cure of *P. vivax* in G6PD normal and G6PD deficient individuals.

RDT formats and protocols can vary. These instructions and the accompanying job aid presented in this manual were designed based on examples of commercially available G6PD RDT products. Therefore, it may be necessary to modify the training and job aid to fit the brand and type of RDT you are using. Particular sections of the training that might need modification include:

- Sections 2.6.3, 2.10, 2.11, and 2.13 on where to add blood and buffer;
- Section 2.16 and Section 5 on interpreting test results.

WHO can assist with these modifications. (Contact Malaria_rdt@who.int)

Table of contents

Glossary of acronyms

AFRO.....WHO – Regional Office for Africa

ALRI.....Acute lower respiratory infection

AQ ..... Amodiaquine

CHW .....Community health worker

DHMT ..... District health management teams

FIND……Foundation for Innovative New Diagnostics

FST…… ..... Fluorescent spot test

G6PD……..…… Glucose-6-phosphate dehydrogenase

GMP……World Health Organization, Global Malaria Programme

NADPH…… Nicotinamide adenine dinucleotide phosphate

RDT..... Rapid diagnostic test

Introduction to trainers on the use of this manual

Purpose

The purpose of this manual is to train health workers to use G6PD rapid diagnostic tests (RDTs) safely and effectively, so as to inform appropriate decision making for *P. vivax* radical cure. The original malaria RDT manual from which this specific manual was adapted, was tested in Zambia with the Zambian National Malaria Control Centre and Zambian community health workers (CHWs).

This manual should be used with the accompanying job aid. The job aid is a set of step-by-step instructions about how to use a G6PD RDT. It contains both words and pictures. You will find a small version of the job aid at the end of this manual. At the end of the training, you should give each participant one or more copies of the job aid to take with them. Participants should use the job aid whenever they perform a G6PD RDT. Without the job aid, the material in this manual will not provide sufficient training. ***You should not conduct the training without the job aid.***

The training takes approximately 3 hours. Based on malaria RDT field testing, this training, ***if used with the accompanying job aid***, should be sufficient to enable most health workers to use G6PD RDTs correctly and safely. However, RDT use should be monitored in the field to ensure good diagnostic practice and blood safety. Further revision of the material may occur following field experience and feedback. We welcome your comments and suggestions. Please send them as an email to: *Malaria_rdt@who.int*. The ideal group size for the training is 10–15 health workers. Conducting the training with more than 15 participants makes it difficult for a single trainer to provide sufficient attention to each participant, particularly during sections 4 and 5. If you plan to use this material with a group larger than 15, it is strongly recommended that you work with one or more assistants who have experience using RDTs and can help you provide one-on-one attention to participants. Even with smaller groups, it would be helpful to have one or more assistants available.

What this manual contains

This manual provides step-by-step instructions for carrying out the training. The table of contents lists each section. The manual also contains a small version of the job aid, a list of frequently asked questions, sample RDTs and answer keys for those samples.

The different styles of type in this manual indicate different things:

Normal type like this is used to explain parts of the training to you, the trainer, and to describe learning objectives, activities, and sometimes specific things you should say to training participants. In some cases, this will include sentences you can read directly to participants.

Blue italic type in a box like this is used to indicate instructions to you, the trainer, about how to manage the training or what to do in a particular situation. These instructions are NOT meant to be read to participants. For example, an instruction of this type might say 'Remind participants to consult national guidelines on use of primaquine for radical cure of P. vivax infection in G6PD deficient individuals.'

Green italic type with a red arrow pointing to it like this highlights areas that may cause difficulty or require special attention. The arrow followed by light italic type may also contain tips about how to resolve or avoid particular problems or overcome barriers.

Purple text like this refers to questions that the trainer can ask the trainees to test their knowledge in important areas.

Text in coloured frames like this relates to topics or activities a trainer needs to cover during each section of the training.

How to use the manual

Before conducting this training, you should have enough experience using the RDT and job aid to feel comfortable carrying out each step of the test safely and correctly. You should also have a good knowledge of national policy on safe radical cure of *P. vivax* in G6PD deficient and G6PD normal individuals. If you have not used the RDT or job aid, you should seek training from someone with experience.

Once you have become comfortable and familiar with the RDT and job aid and understand national policy on radical cure of *P. vivax* infection, read through the entire manual one or more times before conducting the training. Review the learning objectives and presentation material in each section. Notes on common errors and difficulties observed during development of this material are included. You may find these notes useful during your preparation and presentation. In several sections, model answers are given to frequently asked questions from trainees. These model answers are set off from the rest of the text in boxes. You may use them directly as written to work through these important issues with trainees or as a guide to ensure all these important issues are addressed in each section of the training programme. In some cases, it may be appropriate for you to adapt the model answers to reflect national management policy and the specific RDT product in use.

Gather the material and supplies you will need for the training using the list included on page 5. Use the manual as a guide to each section during the training. You are now ready to begin.

What is G6PD deficiency?

G6PD is an enzyme that plays a crucial role in maintaining red blood cell health by generating glutathione during the pentose phosphate pathway which protects erythrocytes from oxidizing agents *(1,2)*. If levels of G6PD are too low, haemoglobin will not bind oxygen and the red blood cell wall will break, resulting in haemolysis. G6PD deficiency is a metabolic disorder arising from genetic defects in the G6PD gene. The deficiency results in a breakdown of red blood cells (haemolysis) when the individual is exposed to particular medications eg. primaquine, pathogens or foods.

Upon exposure to an oxidative substance, symptoms may vary in severity and range from asymptomatic to, fatigue, rapid heartbeat, blood in urine, shock, heart failure, jaundice, and acute haemolytic anaemia (AHA) caused by premature destruction of red blood cells, which can result in death.

G6PD deficiency is the most common inherited sex linked enzyme deficiency, that affects more than 400 million people worldwide, and mostly populations throughout Africa, Asia, The Mediterranean and the Middle East *(4,6)*. Deficiency is inherited on the X chromosome and since males have just one X chromosome, they can be hemizygous G6PD normal or hemizygous G6PD deficient. Full manifestation of the deficiency (<10% of normal G6PD activity) will be seen in male hemizygotes (see Figure 1). *(1)*

Since females have two X chromosomes, they may be homozygous normal, homozygous deficient or heterozygous (see Figure 1). Full manifestation of the deficiency (<10% of normal G6PD activity) will be seen in female homozygous deficient individuals *(1)*. Those who are heterozygous carry deficiency on one gene, but not the other. During lyonization in early development, one X chromosome is switched off in each cell. Which X chromosome is deactivated is random and varies, therefore, heterozygous females can show an intermediate range of deficiency which is usually between 30%-80% of the normal G6PD activity (see Figure 2). The risk of haemolysis is higher in those with severe deficiency.

Plasmodium vivax infection

P. vivax is the most widespread plasmodium species and was responsible for 13.8 million cases of malaria in 2015 *(3)*. While not as pathogenic as falciparum malaria, *P. vivax* is nonetheless responsible for significant morbidity and mortality, and in 2015 was estimated to result in 1,400–14,900

deaths *(3)*. *P. vivax* produces both a blood stage, and a liver stage of infection. In the latter, parasites remain in the liver as dormant hypnozoites which can be reactivated to result in recurrent clinical attacks, unless hynozoites are specifically targeted with 14 day treatment using the 8- aminoquinoline drug, primaquine *(4)*. While primaquine results in radical cure of *P. vivax*, use in individuals with G6PD deficiency can induce severe haemolysis which may result in death *(5)*. The risk of inducing haemolysis while treating *P. vivax* infected patients with primaquine therefore presents a serious public health issue and a potential roadblock for elimination *(6)*. Consequently, G6PD testing is recommended prior to administration of primaquine and forms a crucial component of *P. vivax* control and elimination programs *(7)*.

Methods of G6PD deficiency detection

Traditionally, phenotypic, qualitative diagnostic methods that measure G6PD enzyme activity are used for case management. These tests can distinguish between deficient (<30% or > 30% of normal G6PD activity) but do not reliably distinguish those with 30–80% of normal G6PD activity. The fluorescent spot test (FST) is the most widely used method for qualitative detection of G6PD deficiency. It measures G6PD enzyme activity by detecting the conversion of co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH) produced in the pentose phosphate pathway, into its reduced form. The methodology may vary between manufacturers, but broadly involves the following steps. Patient blood is incubated with glucose-6-phosphate and NADP, which is spotted onto a filter paper then dried. If the patient is not G6PD deficient, NADPH will be produced during the reaction which can be visualized as fluorescence when the filter paper is illuminated under long wave ultraviolet light (UV). If no fluorescence is seen, the patient is concluded to be G6PD deficient *(2)*. Intermediate deficiency can also be detected using this method. FST has its drawbacks, it is expensive, requires skilled technicians, specific equipment and access to a cold chain *(2)*, which generally makes this technique unsuitable for use in peripheral health care facilities in remote field settings.

G6PD detecting RDTs have been developed more recently. RDTs are lateral flow chromatographic tests that offer rapid qualitative detection using inexpensive, user friendly methodology that can be successfully followed after training. RDTs can be tested from a finger prick sample, do not require specific equipment and can be performed at point of care to allow prompt determination of G6PD deficiency status prior to safe administration of treatment. The RDT is comprised of a nitrocellulose strip housed inside a plastic cassette. Blood, followed by buffer are added to the RDT, then a 10 minute incubation step is observed to allow the sample to wick the length of the nitrocellulose strip. Normal G6PD activity is indicated when nitro blue tetrazolium (impregnated on the nitrocellulose strip) is reduced to formazan which is purple. G6PD deficiency is identified through lack of a colour change (the strip remains white) *(8)*.

Currently *P. vivax* patients are often treated with primaquine without knowing their G6PD deficiency status. This carries clear risks of inducing acute haemolysis in those who are G6PD deficient. If G6PD testing is not available, a decision to administer primaquine should be based on a risk–benefit assessment.

Why are job aids and training necessary?

The instructions for use provided by many RDT manufacturers are sometimes confusing and inadequate.

This manual and the accompanying job aid contain the information health workers need to use RDTs safely and effectively. The material included here can help improve the quality of diagnosis and the safety of both health workers and patients. However, follow-up supervision and monitoring of diagnostic practice and blood safety in the field is an essential part of RDT-based diagnostic policy.

The material included here does not address drug therapy in detail. Rather, it assumes that health workers and other trainees have received orientation on local and/or national drug policy, and on the side-effects and precautions of administration of primaquine in G6PD deficient and G6PD normal individuals through some other module or course.

It is recommended that a set of pre-prepared RDTs with G6PD normal and G6PD deficient results also be used during the training. These may be prepared with known deficient and normal blood or alternatively, manufacturers may sell controls (normal, intermediate and deficient) separately.

We welcome your comments and suggestions, based on your own experience using these tools, and will be happy to consider incorporating G6PD genotypes and phenotypes into future editions. For more information and to provide feedback, please contact:

WHO Global Malaria Programme
Malaria_rdt@who.int

Figure 1 – Genetics and biochemistry of G6PD deficiency

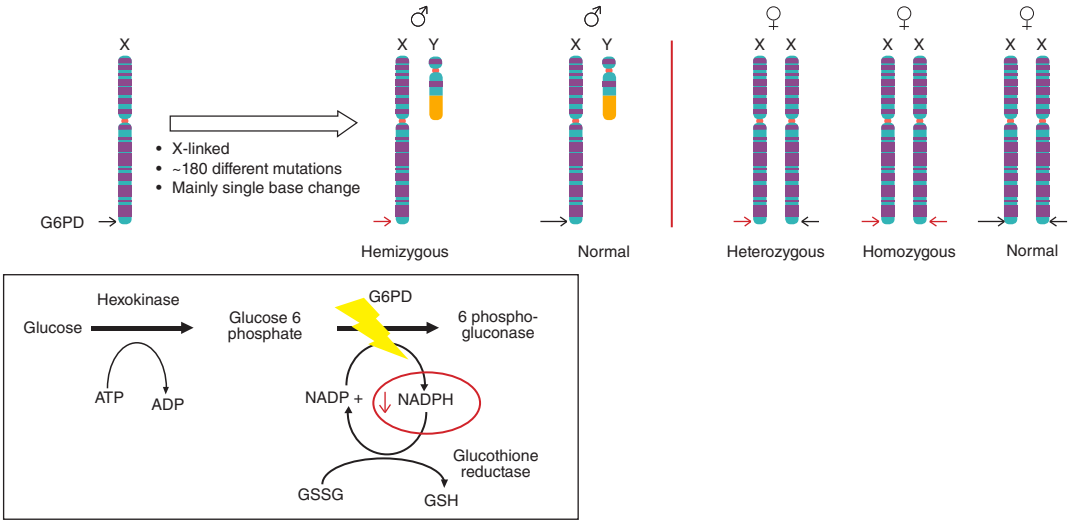
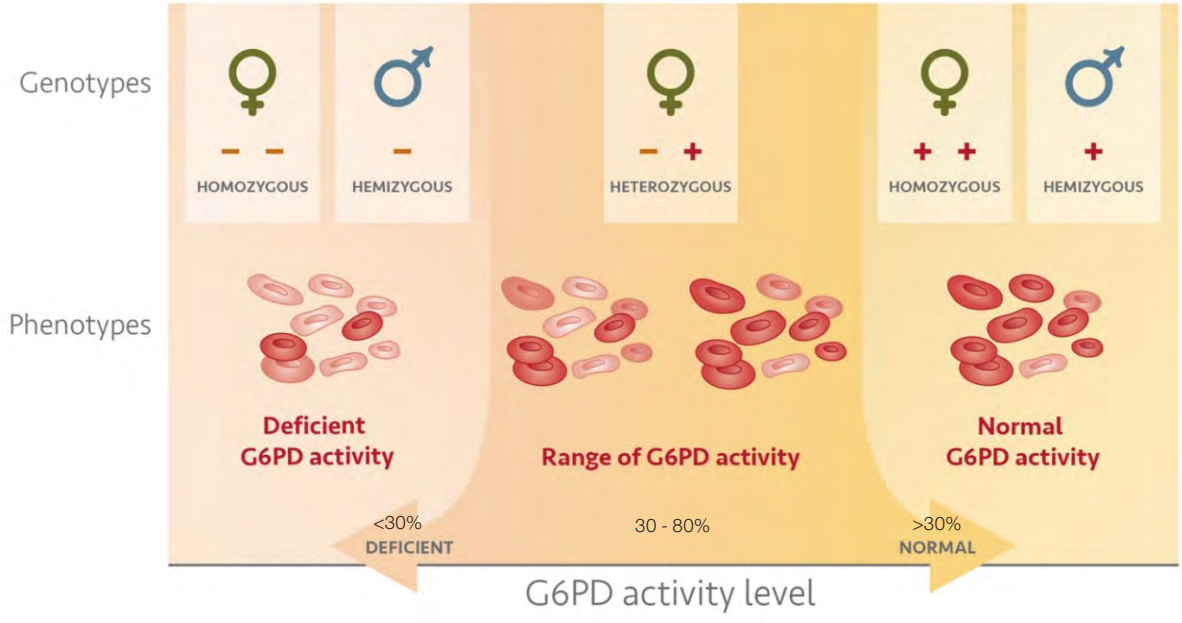


Figure 2 – G6PD genotypes and phenotypes



Materials and supplies needed for training

Item	Notes
1. RDT test packets	At least 2 per participant plus some extras to have in reserve in case some participants need additional practice. You will also need 1 or 2 packets to use yourself during the orientation.
2. Disposable examination gloves	At least 2 pairs per participant, 2 pairs each for you and anyone who will be assisting you during the training, and some extras to keep in reserve. It is not necessary to use sterile gloves to prepare an RDT.
3. Alcohol swabs	2 per participant, 2 for you to use during demonstrations, and several extras to keep in reserve. Alcohol swabs are often included in each box of RDTs. If alcohol swabs are not available, it is also possible to use cotton wool and bottles of 70% alcohol or another appropriate disinfectant, but pre-packaged swabs are easier to handle and more convenient.
4. Sterile disposable lancets	One lancet per RDT and additional ones for demonstration and failed attempts. These are also often included in each box of RDTs or may be obtained separately.
5. Sterile gauze pads or cotton wool	2 per participant, 2 for you to use during demonstrations, and several extras to keep in reserve.
6. Buffer	One dropper bottle of test buffer for every 2 or 3 participants. 1 or 2 buffer bottles are generally included with RDT kits by the manufacturer. Participants will not use an entire bottle during training, but logistics are much simpler if you have several bottles rather than just 1 or 2. Buffer left over from previous training sessions can be used but only if the same lot number of RDTs are used.
7. Sharps disposal bins	Ideally, you should have one sharps bin for every 2 or 3 participants. At a minimum, you will need enough sharps bins that each participant has one within easy reach so that they can dispose of their blood lancets and blood-transfer devices immediately after using them and before setting them down on the work area.
8. General disposal containers	A sufficient number of potentially infectious and general waste containers for all participants to dispose of their gloves, test cassettes, wrappers, swabs, and other non-sharps material.
9. Chairs and tables	One chair for each participant, and work benches or tables.
10. Easel and pad / white board or LCD projector	One easel pad or white board or LCD projector if reliable electrical supply.
11. Pens / markers	Felt-tipped pens for use on easel pad or white-board markers for use with such boards: at least one black and one red.
12. ARVs	Two to three initial doses of anti-retroviral (ARV) post-exposure prophylaxis to reduce HIV/AIDS risk if someone is pricked by a lancet that has already been used by another person. It is important to emphasize to participants that this is good clinical practice.
13. RDT colour plates, quizzes	Colour plates of RDT results (and preferably pre-prepared RDTs), RDT quizzes, and RDT instruction sheet for every participant.
14. Work space	Sufficient work area for each pair of participants to practice performing RDTs on each other. This could be one small table per pair, several larger tables with one pair working at each end and one pair in the middle, or sufficient counter space.

Section 1 Introduction *(10 minutes)*

Learning Objectives

1. Participants will be able to describe a rapid diagnostic test and why the malaria control program has elected to use G6PD RDTs in the country/district.
2. Participants will be able to describe appropriate actions for G6PD deficient and G6PD normal results.

Topics to cover

- What are RDTs?
- Why are they important for safe radical cure of *P. vivax* in G6PD deficient patients?
- Limitations of RDTs
- Actions for G6PD normal and G6PD deficient RDT results

You may want to have a large photograph or drawing of an RDT to show participants while you present the material in this section. In the next section, you will open and show participants a sample of the actual RDT you will be teaching them to use.

— 1.1 —

What are RDTs?

Rapid diagnostic tests or RDTs can be used to detect pathogens, chemicals or enzyme deficiencies. Specifically, in this case, they are a way to test whether a person with vivax malaria has G6PD deficiency, which will determine their treatment programme for radical cure of *P. vivax*.

To perform an RDT, blood is added to a designated well in the cassette, followed by buffer which is added to a separate well. The RDT is incubated for a few minutes then G6PD status can be determined. Normal G6PD activity is indicated by the production of a purple colour which is produced when nitro blue tetrazolium, which is embedded on the RDT test strip, is reduced to formazan which is purple. In G6PD deficient patients, there is no colour change and the background of the test strip remains white. Cases of faint colour change can occur and manufacturers advise to classify these as G6PD deficient.

— 1.2 —

Why are RDTs important for identifying G6PD deficiency?

- In the past, the following approaches to diagnosing G6PD deficiency and treating *P. vivax* infection with primaquine have been adopted :
  - No G6PD testing or primaquine treatment. Treatment with primaquine has been withheld due to prevalence of G6PD deficiency, absence of field deployable G6PD detection methods, limited availability of primaquine or lack of ability to monitor and respond to adverse effects of primaquine.
  - No G6PD testing but primaquine has been administered (various doses have been used). Local population prevalence of G6PD deficiency has previously been used to estimate individual G6PD status. This approach carries clear risks to the patient if they are G6PD deficient.
  - G6PD testing, followed by appropriate primaquine administration is the ideal approach to case management. Previously FST has been recommended as the most applicable method for field detection of G6PD

deficiency, but has numerous drawbacks such as the need for specific equipment, trained personnel and a cold chain. Use of a field deployable diagnostic method will expand the ability to test and treat more people especially in remote health facilities.

- RDTs are a simple and fast way for health workers to test for G6PD deficiency in a patient's blood at the bedside. RDTs can help identify patients with G6PD deficiency, so appropriate primaquine drug regimens may be safely administered and carefully monitored. Identifying and treating such patients will improve morbidity and mortality while reducing the *P. vivax* transmission reservoir, which will help control and elimination efforts.
- RDTs can give results in about 10 minutes (check instructions for use), so a patient can begin treatment immediately.
- RDTs do not require any expensive or complicated equipment. Most people can learn to use RDTs in just a few hours. Today's training will be enough for most of you to learn how to diagnose G6PD deficiency safely and effectively with an RDT.
- Some G6PD RDTs do not require a cool chain and can be stored and used between 18–32°C.

> ***Note to trainer:***
> *This answer may be shortened to address only the specific RDT you are using.*

— 1.3 —

What are some limitations of RDTs?

1. The result of a G6PD RDT is that of an enzymatic reaction. Enzyme reactivity is influenced by temperature and therefore, manufacturers instructions for use must be strictly respected. Currently available RDT are recommended for use between 18°C and up to either 25°C or 32°C. If RDTs are used at higher temperatures, it could result in deficient patients being incorrectly recorded as normal.
2. As illustrated in Fig. 2, RDTs cannot distinguish females with normal G6PD activity from those with intermediate activity due to being heterozygous for G6PD deficiency. This could result in some heterozygous females with intermediate activity being categorized as normal, and consequently being treated with primaquine. This could potentially result in serious adverse events, including acute haemolytic anaemia; however, there is limited data to determine the risk.
3. If the G6PD RDT does not have a control line then to be confident that the sample has migrated the full length of the strip and that the RDT has worked as expected, it is best to watch the sample flow to the end of the results window to ensure migration was not incomplete or interrupted.
4. RDTs can be damaged by heat and humidity, and must be stored at the manufacturer recommended temperature range. RDTs should not be removed from their sealed package until right before you are ready to use it. If a package has been open for some time before the RDT is used, the RDT may be damaged by heat or humidity and give an invalid (false) result. You should discard this package and use another, unopened, package.
5. To work properly, RDTs need blood and a chemical called 'buffer'. Adding too much or too little blood or buffer can cause the test to give an invalid result or be difficult to read. Adding blood and buffer in the wrong place can also cause an invalid result. Substituting the buffer with another liquid can result in false results, ensure that only the supplied buffer is used with the RDT. This training will show you how much blood and buffer to use and where to add them. You will practice using RDTs to test one another for G6PD deficiency. Pay careful attention during the training, and use the job aid during the practice session, so your tests give correct results.
6. Manufacturers should provide colour charts to allow for objective interpretation of results. This is particularly important in the case of faint background colour changes. Generally, it is recommended to classify these cases as deficient and avert risk of severe hemolysis.

— 1.4 —

Actions for G6PD normal and G6PD deficient results

Note to trainer:
This section provides a very brief summary of general treatment policy based on G6PD RDT results, and should be modified to fit the specific product in use. It is meant to reinforce instruction on how to prescribe primaquine. It is NOT meant to provide a full explanation of primaquine treatment policy. ***Details should be adjusted to reflect your country's national treatment policy.***

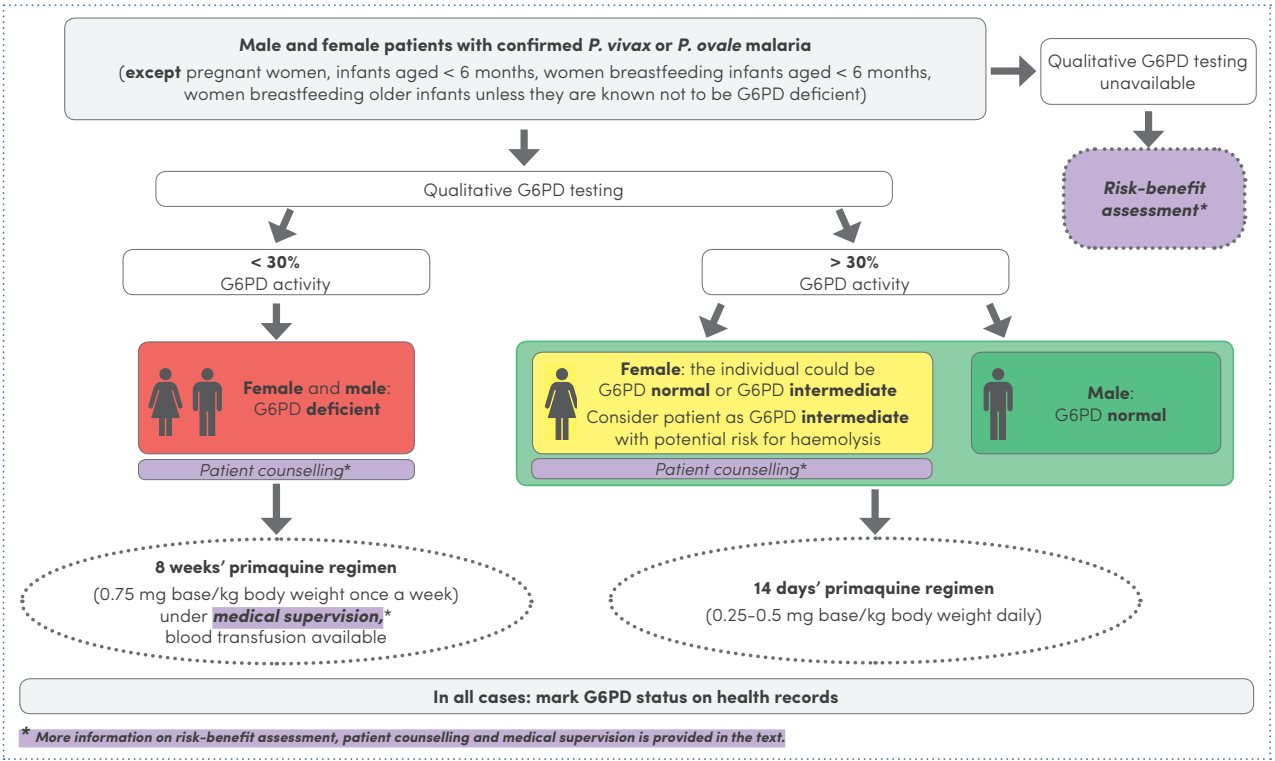
1.4.1 After you use the G6PD RDT and get the result:

Refer to the national guidelines to confirm the treatment regimen for radical cure of *P. vivax* in **G6PD deficients and G6PD normal patients.**

Figure 3 below details the general structure of a G6PD testing, counselling and treatment programme, depending of the patient G6PD status.

Please note: these are general guidelines, your country's national treatment policy must be followed.

Figure 3. Algorithm for qualitative point-of-care testing and safe administration of primaquine to prevent relapse of *P. vivax* or *P. ovale* malaria in male and female patients



Source: Testing for G6PD deficiency for safe use of primaquine in radical cure of *P. vivax* and *P. ovale*: Policy Brief, WHO/HTM/GMP/2016.9, World Health Organization, 2016

Section 2 How to use an RDT *(45 minutes)*

Learning Objective:

Participants will have a general understanding of how a G6PD rapid diagnostic test is performed.

Activities to cover:

a. Perform the test on a volunteer with all participants watching;

b. While performing the test, explain in detail how to carry out each test step;

c. Use the job aid as a visual aid for describing and explaining each test step.

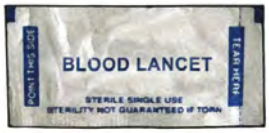
This demonstration works best if participants are watching from close by. If you are conducting the training in a large room or if some participants are sitting more than 2–3 meters away, ask everyone to gather around the table where you are working so that they will be able to see clearly. Participants will be able to move in more closely and see more clearly if they are standing, rather than sitting, for this section of the training.

Participants learn more rapidly and remember longer if they participate actively. As the trainer you should carry out the demonstration in this section to show how to perform the test correctly and safely. However, you should involve participants as much as possible in the demonstration by asking them to explain why you are doing certain steps in a certain way. For instance, when you put on gloves, you can ask, 'Why is it important to wear gloves during this test?' In the guide below, we note many opportunities where you can involve participants by asking them a question or asking them to do a particular task. If you think of additional ways to involve participants, don't hesitate to use them.

— 2.1 —

As shown on the job aid, assemble all the supplies you will need, including:

- A new, unopened test package
- A new, unopened alcohol swab
- A sterile lancet (new and unopened)
- Buffer
- A new pair of disposable examination gloves
- A watch or clock to use as a timer
- Pencil
- Blood transfer device



- A sharps disposal container
- A non-sharps disposal bin for potentially infectious and one for general waste

— 2.2 —

Place all these supplies on a table where they will be visible to all participants.

Point out the list of supplies on the job aid, then point to each one on the table and identify it for the participants.

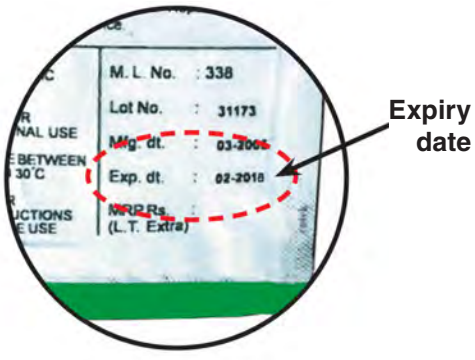
— 2.3 —

Ask for a volunteer from among the participants.

This person will act as the 'patient'. He or she will help you demonstrate how to perform the RDT.

— 2.4 —

Explain the importance of the expiry date.



2.4.1 Point out the expiry date on the test package, but do not read the date. The product is considered to be acceptable for use until the end of month shown.

2.4.2 Pass the test package around and ask each participant to look at it.

2.4.3 Once all participants have had a chance to look at the expiry date on the package, ask them: **'What is the expiry date?'**

2.4.4 If the first person to answer gives the correct expiry date, ask the others: **'Does anyone disagree?'**

If anyone suggests a different date, have the participants discuss among themselves which date is correct and why. Once the participants reach a consensus (or once it becomes clear that the participants cannot resolve the disagreement), tell them the correct date, point out its place on the package again, correct any mistakes and answer any questions.

2.4.5 If the first person to answer gives the incorrect expiry date, ask the others: **'Does anyone disagree?'**

Have the participants discuss among themselves which date is correct and why. Once the participants reach a consensus (or once it becomes clear that the participants cannot resolve the disagreement), tell them the correct date, point out its place on the package again, correct any mistakes and answer any questions.

Field tests show that many health workers do not immediately understand the concept of an expiry date, naturally enough since their environments do not commonly have such dates. Also, many have trouble finding the expiry date on the package or forget to read it before carrying out the test. You should emphasize repeatedly the importance of checking the expiry date and not using an expired RDT. Ask questions from the participants and encourage them to ask questions until you are sure everyone understands.

— 2.5 —

Wash your hands and put on a new pair of examination gloves.

As you are putting them on, ask participants: **'Why is it important to wear gloves when doing the test?'** Be sure someone mentions the following two points:

2.5.1 Protect health workers from possible infection with blood-borne diseases, including HIV-AIDS.

2.5.2 Protect patients from possible infection with blood-borne diseases, including HIV-AIDS.

— 2.6 —

Open the test package and remove the contents. (Contents of the cassette/test packaging may vary.)

As you remove each item, hold it up so that everyone can see it. Describe what each item is and explain how it is used:

2.6.1 The blood-transfer device — (a) capillary tube, (b) straw, (c) loop, (d) pipette, type 1, (e) pipette, type 2, (f) inverted cup, or other — is used to collect blood and transfer it to the test cassette.

a.

b.

c.

d.

e.

f.

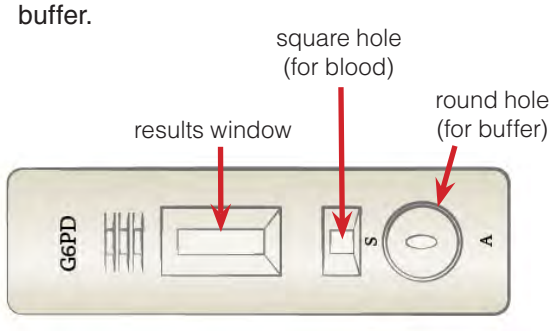
2.6.2 The desiccant sachet protects the test from humidity before the package is opened.

Once the package is opened, the desiccant sachet serves no purpose and should be discarded. It may be harmful if swallowed, so it should be kept away from children. Refer to the instructions for use, if the desiccant contains beads that change colour when exposed to moisture, check the desiccant for presence of a colour change before using the RDT. If beads have changed colour, dispose of the RDT and select a new test.

2.6.3 The test cassette is used to conduct the test. Pass the cassette around and ask everyone to look at it. Explain the holes and the markings and what each one means:

For example:

- The square hole labeled 'S' is where you add the blood.
- The round hole labeled 'A' is where you add the buffer.



- The rectangular hole is the results window where you read the test results.

— 2.7 —

Write the patient's name on the cassette.

Explain to participants why it is important to write the patient's name on the cassette before beginning the test:

There will probably be times when you have many patients waiting to be diagnosed. You won't be able to wait to get each patient's result before testing the next one. If you are testing several people one after another, you will need to have their names written on their cassettes so you don't run the risk of mixing up one person's results with those of another. Even when you have only one patient to test, it is good practice to write his or her name on the cassette so you develop the habit of doing it and don't forget to do it when you are busy and have many patients.

— 2.8 —

Open the alcohol swab. Clean the patient's 4th finger.

Clean the patient's 4th finger. Explain these important steps in using the alcohol swab:

2.8.1 Ask the patient: **'Are you right-handed or left-handed?'** If the patient is right-handed, choose the 4th finger on their left hand.

If the patient is left-handed, choose the 4th finger on their right hand. This will cause the least inconvenience to patients if the pricked finger becomes sore.

2.8.2 The 4th finger is preferred because for most people it is the least-used finger.

Pricking this finger will cause the least inconvenience for most patients because if it becomes sore, it will not interfere with their work. Also, since it is least used, it may be less likely to become infected later. Further, the skin on the 4th finger may be thinner. Other fingers may be used if necessary.

Be sure that participants understand that by the 4th finger, we mean the one closest to the little finger (see illustration). In some places, people count fingers beginning with the little finger and ending with the thumb instead of the other way around. If some of your participants are accustomed to counting this way, they will identify the index finger as 4th finger.

2.8.3 After cleaning the finger with the alcohol swab, it must be allowed to ***air dry.***

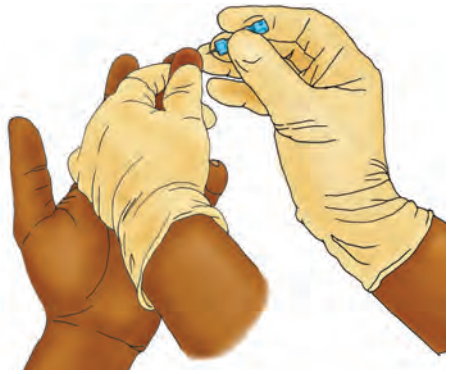
The finger ***must not*** be dried by blowing on it or wiping it with a piece of cloth or paper. Do not allow the participant to blow on it. Ask participants: **'Why must you not blow on or wipe the finger once it is cleaned?'** Make sure they understand that blowing on or wiping the finger means it will no longer be clean.

2.8.4 After using the alcohol swab, place it on its wrapper and set it aside on the table. You can use it again if you don't have a cotton swab, to stop the bleeding after you collect the 'patient's' blood.

— 2.9 —

Once the patient's finger is dry, open the lancet.

Prick the patient's finger, preferably towards the side of the pulp (ball) of the finger. Pricking the midline or tip is more painful. Check to be sure the finger-prick will produce enough blood, then immediately discard the lancet in the sharps container. Remind participants that every time they use a lancet, they must take ***all*** of the following steps to ensure blood safety:



- Discard the lancet in an appropriate sharps container ***immediately*** after using it. Wipe away the 1st drop of blood with a sterile gauze pad or piece of cotton wool, then discard this in the infectious non-sharps waste bin. The 2nd drop of blood is tested on the RDT.
- ***Never*** set the lancet down before discarding it.
- ***Never*** discard the lancet in a non-sharps container.
- ***Never*** use a lancet on more than one person.

You will need to make these points quickly so your volunteer 'patient's' blood does not coagulate before you can collect it. You will have time to make them again and discuss them in more detail when you present the next section.

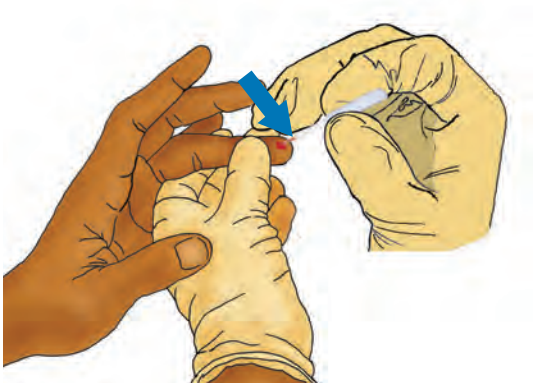
> ***Note to trainer:***
> *Post-exposure prophylaxis for HIV: When lancets are used carefully and strictly according to the above instructions, there is minimal risk of finger-prick injury to the user or other people. However, should an accident occur and a person receives a finger prick with potentially infective material, it is essential that the current national policy for post-exposure prophylaxis be followed promptly. Trainers should familiarize themselves with this policy prior to conducting the training, and ensure that the trainees are fully aware of the policy and where they can access PEP if required.*

— 2.10 —

Demonstrate how to collect the droplet of blood using the blood-collection device included with the RDT you are demonstrating (two examples are provided below):

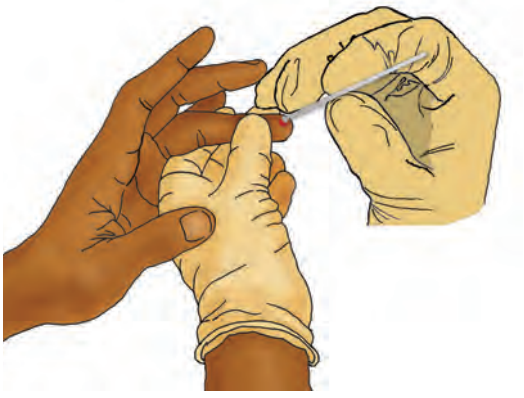
Pipette:

Collect blood until the 2µL mark on the pipette as follows (always confirm with the product instructions).

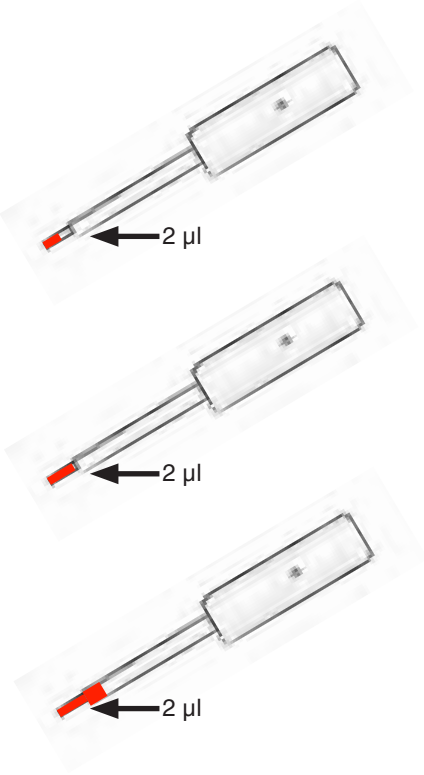


Inverted cup:

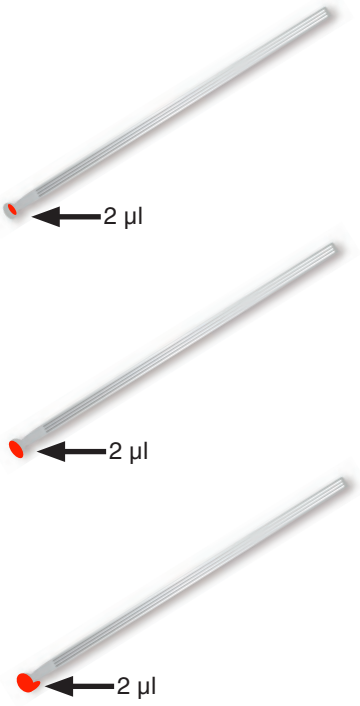
- Ensure a good size drop of blood is on the finger before collecting with the device.
- Place the tip of the inverted cup onto the drop of blood by holding the inverted cup at a 25 degree angle.
- Explain that the blood will then be absorbed automatically.
- Lift the device when the cup is filled with blood.
- Explain it is important not to make abrupt movements or touch anything with the transfer device during blood transfer, as this could lead to spillage of the blood.



Pipette:



Inverted cup:



— 2.11 —

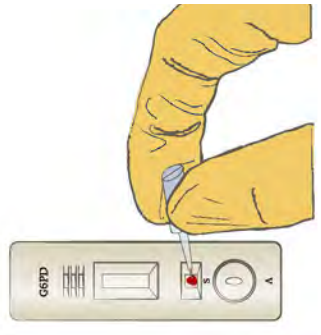
Use the blood transfer device to add the drop of blood to sample window (square hole with letter S).

2.11.1 Explain that the blood needs to reach and be absorbed by the pad at the base of the hole.

If the blood is mostly deposited on the plastic edges of the well, but does not reach the pad, the test will not work correctly.

2.11.2 Explain and demonstrate how to introduce the blood into the hole.

- Pipette: Position the end of the pipette into the sample well in the RDT. Then cover and press the hole at the top of the pipette to release the blood into the sample well.



- Inverted cup: Hold the cup vertically and place against the sample well until the blood is absorbed.

Some RDT users have been observed sucking blood into the blood-collection device. This is incorrect and dangerous. Remind participants that they should NEVER suck blood into the blood-collection device.

2.11.4 Hold up the test so that all participants can see how you have added the blood to the test and how the pad is absorbing it.

Show that the blood is on the pad and not just on the plastic walls of the cassette.

— 2.12 —

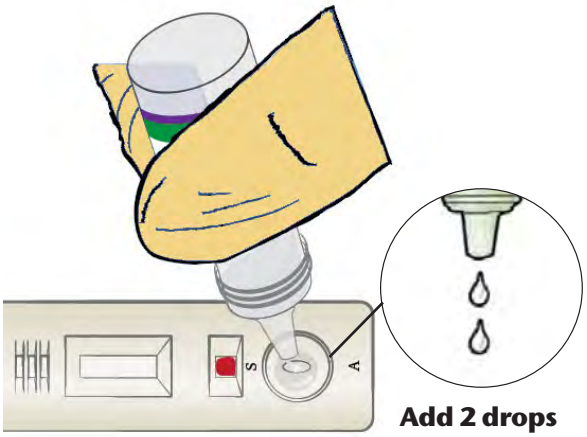
Explain and demonstrate how to discard the blood-collection device after use.

Participants should understand that they must discard the blood-transfer device in the sharps box or potentially infectious waste container, immediately after they transfer the blood to the test cassette. They should not set it down on the table or elsewhere before discarding it.

➢ *In field trials carried out during the preparation of this manual, some health workers had trouble handling the lancet and the blood-transfer device because their hands were shaking. Many were nervous because they had never done a finger-prick. Others had trouble manipulating the blood-transfer device into the small hole. If you observe this during section 4 when participants are practicing RDT use, encourage the participant to relax and reassure them that it becomes easier with practice.*

— 2.13 —

Explain and demonstrate how to add buffer to the cassette (round hole with letter A).



2.13.1 Ask participants: **'Where do we add the buffer?'** Remind them that the buffer must be added to the correct well (hole).

2.13.2 Explain that they need to add exactly the correct number of drops of buffer. Ask them: **'How many drops of buffer do we add to the cassette?'**

Tell them to watch closely as you add the buffer. Hold the bottle vertically (see illustration) — this ensures the correct drop size. To reinforce the correct number of drops, it may help to have participants count them out loud as you add them.

— 2.14 —

Wait for the correct duration of time (*10 minutes*) after adding buffer before reading test results.

- After adding buffer, ask participants: **'What is the time now?'**
- Ask participants to write down the time of day on a note pad or piece of scrap paper.
- Ask for a volunteer to be responsible for telling the group when the correct time (e.g., 10 minutes) has passed. Make sure this volunteer has access to a watch, clock, or other timer.
- Ask participants: **'What time will it be when the correct time has passed?'** Ask them to write this time down on their note pad or scrap paper next to the start time.
- Once participants have recorded the time at which they can read the test results, have them look at the cassette.
- Point out to them how the blood is beginning to wick up the strip, disappearing from the square hole where it was added and beginning to appear in the results window.
- Explain that the blood will eventually disappear from the results window as well, leaving either presence or absence of pigment indicating if G6PD deficiency is present or not.
- It is not necessary for participants to understand every detail of how the test works. But understanding the basic idea of how the buffer washes the blood up the test strip will help them understand why they need to wait the correct time before reading the test results: explain that if there is too much blood left in the results window, they have not allowed enough time, and they will not be able to see the results clearly.

— 2.15 —

Remove and discard your gloves at this time.

- Explain to participants that once the buffer is added to the cassette, gloves are no longer needed for their or their patient's safety.
- To avoid possible contamination, the used gloves should be discarded in the potentially infectious waste container or general waste if the former is not available before the health worker does anything else.

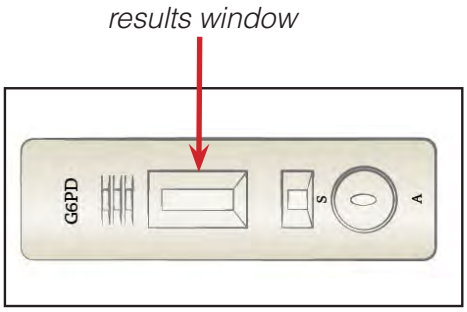
- If a health worker is going to begin a new RDT to diagnose another patient while waiting for the first patient's results, he or she must put on a new pair of gloves.
- If the health worker will do another activity that does not involve handling blood or bodily fluids while waiting for the first patient's results, he or she should not wear gloves.
- In either case, the used gloves should be discarded before moving on.

Keeping the gloves on may result in unseen blood contamination of pens, paper and other materials, and potential ingestion of blood-borne viruses.

— 2.16 —

Reading test results 1: The different possible results and what they mean

Present this step while you are waiting for the correct duration of time before reading test results.



■ Use the easel pad or white board and magic markers to draw all 3 possible results:

1. A distinct purple colour in the results window indicates normal G6PD enzyme activity.

2. No colour change or a very faint purple colour indicates presence of G6PD deficiency. A borderline result is read as deficient (*8*).

3. No migration, or incomplete migration of blood indicates an invalid result.

■ Explain that an invalid result means the RDT is damaged and the results may be incorrect. Ask participants: 'What should you do when this happens?' (Correct answer: Discard the cassette. Open a new cassette and repeat the test using the new cassette.)

■ Ask participants: **'What should you do in case of a result, indicating the patient has G6PD deficiency?'**

(Correct answer: Follow national guidelines on radical cure of *P. vivax* in G6PD deficient individuals.)

■ Ask participants: **'What should you do in case of a result, indicating the patient has normal G6PD activity?'**

(Correct answer: Follow national guidelines on radical cure of *P. vivax* in G6PD normal individuals.)

■ Discuss the national *P. vivax* radical treatment policy in G6PD deficient and G6PD normal patients with participants.

— 2.17 —

Reading test results 2: What is the actual result of the RDT you have just demonstrated?

■ Once the correct amount of time has passed, you should proceed to reading the actual test results.

■ First, read the result yourself, but DO NOT yet tell the result to participants.

■ Pass around the cassette. Ask all participants to look at the cassette and, without speaking, write down what they think is the correct diagnosis on a piece of paper. Each participant should decide the result himself or herself. Participants should not discuss their interpretation of the test with each other.

■ Once all participants have seen the cassette and written down their result, ask for a show of hands:

- **'Who thinks the result is G6PD deficient? Why do you think the result is G6PD deficient?'**
- **'Who thinks the result is G6PD normal? Why do you think the result is normal?'**
- **'Who thinks the result is invalid? Why do you think the result is invalid?'**

• Explain the correct result. Explain why that result is the correct one.

■ Pass around the cassette again. Ask those who answered incorrectly to look carefully at the test and note why the actual result is correct e.g., if people said G6PD normal, but the purple pigment is very faint meaning the real result is borderline or intermediate, and should be interpreted as G6PD deficient, or if people said G6PD deficient but the blood did not fully migrate across the test window meaning the real result is invalid.)

- Explain that if they try to read the result too early, the sample may not have had sufficient time to migrate across the strip and/or for the enzymatic reaction to occur and produce a colour change.

Reading the result past the recommended reading time can lead to G6PD deficient cases being classifed as normal because over time there is enough G6PD present to evenutally react with the substrate and cause a colour change. Similarly, performing the test at high temperature will accelerate the enzymatic reaction and may lead to normal test results in G6PD deficient patients.

- Ask participants: 'How many of you own a watch, clock, or timer?' Ask them: **'How will you measure the correct time when you are working in your health facility?'**

Section 3 How to take a finger-prick blood sample

(15 minutes)

Learning Objective:

Participants will be able to demonstrate the correct technique for drawing blood safely and effectively with a finger-prick, including the reasons for universal precautions.

Health workers who have never collected a finger-stick blood sample need specific instructions and practice to master the technique. The purpose of this section is to demonstrate how to handle the lancet and collect the blood sample effectively while minimizing pain for the patient and minimizing infection risk for both the patient and health worker.

As in the previous section, participants should watch from close by. If you are in a large room, or if some participants are sitting more than 2–3 metres away, ask them to gather around the table so that they will be able to see clearly.

Activities to cover:

Ask for a new volunteer from among the participants to play the role of 'patient.' Using this volunteer, describe and demonstrate the elements of effective finger-pricking to collect a small quantity of blood.

— 3.1 —

Put on gloves before beginning. Use a new pair of gloves for each patient. Do not re-use gloves.

- Ask participants: **'Why is it important to wear gloves?'** Answer: To protect themselves and the patient against potential infection with blood-borne disease.
- Ask participants: **'Is it OK to use gloves on more than one patient? Why?'** Answer: Gloves should never be used on more than one patient because they could expose the second patient to an infection from the first. They must use a new pair of gloves with each patient.

— 3.2 —

Select an appropriate finger (i.e., 4th finger of left hand—explain which is the 4th finger using the job aid to illustrate).

- Ask participants: **'Why should we use the 4th finger?'** They should remember from the previous section that people generally use their 4th finger less than the others. We select it for drawing blood because it minimizes inconvenience to the patient.
- Instruct participants: **'Identify your 4th finger.'** They should remember from the previous section that the 4th finger is the ring finger, not the index finger: we count from the thumb to the little finger, not vice-versa.
- Ask participants: **'Which of the patient's hands should you use to draw blood?'** They should remember from the previous section to use the left hand if the patient is right-handed and the right hand if the person is left-handed.

— 3.3 —

Clean the finger with alcohol to prevent infection.

- Demonstrate and explain appropriate cleaning technique: wipe the entire first joint of the finger with the alcohol swab, paying particular attention to wetting the pulp (ball) and sides of the finger tip.

— 3.4 —

Allow the finger to air dry.

- Ask participants: **'Is it OK to blow on the finger to dry it more quickly?'** Answer: No, this would re-contaminate the finger with germs from the breath.
- Ask participants: **'Is it OK to dry the finger with a cloth or piece of paper (e.g., toilet tissue)?'** Answer: No, this would re-contaminate the finger with germs from the cloth or paper.

— 3.5 —

Open the lancet immediately prior to use. Once the lancet is open, do not set it down.

The following mistakes are common among people with little or no experience handling a blood lancet:

- The provider opens the lancet before cleaning the finger, then sets the lancet down on the table while cleaning the finger.
- The provider opens the lancet before cleaning the finger, then tries to hold on to the lancet while cleaning the finger.
- The provider opens the lancet without paying attention to where the point is located, thus exposing himself or herself to an accidental finger-prick.

— 3.6 —

Prick the side of the finger (not directly on the pulp or ball). Stab firmly and deep enough to draw an adequate amount of blood.

The following mistakes are commonly observed among health workers inexperienced at taking finger-prick blood samples. The first two generally happen because the provider is nervous about pricking the patient:

- The provider stabs too lightly and either fails to puncture the skin entirely or creates a puncture too small to collect a sufficient amount of blood. This results in the need for a second or third or fourth prick.
- Rather than stabbing, the provider places the point of the lancet on the patient's finger and attempts to push it through the skin. This is ineffective, especially with patients whose fingers are calloused. It is more painful to the patient than a quick, firm stab.
- The provider stabs the centre of the finger tip rather than the side or stabs too close to the nail bed.

— 3.7 —

If blood is insufficient after first prick, try the following.

- If insufficient blood after pricking the finger, try to produce more blood by gently pushing down towards the tip of the finger by squeezing along the finger towards the prick. More blood will be generated by this simple method rather than pressing the ball/pulp of the finger from the bottom or pressing at the two sides of the fingertip. A method such as mentioned above can be used to draw sufficient blood before pricking the finger a second time.

— 3.8 —

Dispose of the used lancet safely in the sharps box. Do not set it down before disposing.

- Inexperienced providers often set the lancet down on the table before discarding it. Ask participants: **'Why should you NOT set down the lancet on the table?'** Answer: It increases the risk that they will prick themselves when they pick the lancet back up, thus exposing themselves to potential infection from the patient. Also, it may be forgotten and put others at risk later.
- Inexperienced providers sometimes forget to discard the lancet in an appropriate sharps container and instead discard it in a normal waste container. Ask participants: **'Why must you use a sharps container and not discard the lancet with normal waste?'** Answer: If they discard the lancet with normal waste, they put themselves and any others who handle the waste at risk of infection through a puncture wound.

- Emphasize the importance of using an appropriate sharps container and remind participants of your health system's policy for disposing of full sharps containers.
  - In some health systems, the district health management team (DHMT) collects used sharps containers from health workers and incinerates them.
  - In others, the DHMT instructs health workers to dispose of sharps in a pit toilet.
  - Ask participants: **'Why must you NOT dispose of sharps in a normal rubbish pit?'** Answer: Because children and others could come into contact with contaminated sharps there.
- All end-users of RDTs should be told what to do in the event of accidental lancet/needle-stick injury in accordance with the national policy on post-exposure prophylaxis.

Section 4 Perform a G6PD rapid diagnostic test

(45 minutes)

Learning Objective:

Participants will develop the skill to safely and effectively perform a G6PD RDT using the job aid as a guide.

Activities to cover:

a. Divide the trainees into groups of 2 or 3 depending on the number of trainees. (If you have 10 or fewer trainees, have them work in groups of 2; if you have more than 10, have them work in groups of 3.)

b. Within each group, participants take turns performing an RDT on one another using the job aid as a guide.

c. Each group member must perform ***correctly*** a ***minimum*** of one RDT. Correct performance means completing ***all*** crucial steps correctly.

Each group will need the following supplies:

- 2 new sealed RDT test packages *per participant* (that is, a total of 4 packages for groups of 2 health workers or a total of 6 packages for groups of 3; similar calculations must be made for the next few supply items)
- 2 new pairs of examination gloves per participant
- 2 alcohol wipes per participant
- 2 lancets per participant
- 1 bottle of buffer
- 1 pencil per participant
- 1 sharps disposal bin
- 1 general disposal container and 1 potentially infectious waste container
- 1 clock or watch (if one of the participants in each group has a watch, ask them to allow group members to use it as their timer)
- 1 copy of the job aid
- 2 sterile gauze pads or 2 pieces of cotton wool

Specific steps:

— 4.1 —

Distribute supplies to each group of 2 to 3 participants.

— 4.2 —

Instruct participants within each group to take turns performing the RDT on each other.

Each member of the group should perform one RDT. Next, after each participant has completed one RDT, the group should begin again until each participant has performed the whole procedure at least twice.

— 4.3 —

Instruct participants to FOLLOW THE JOB AID as they complete their RDTs.

— 4.4 —

Instruct participants to KEEP THEIR CASSETTES once they have finished so you can check the results in the next section. DO NOT DISCARD cassettes at this stage.

— 4.5 —

Once a participant has added buffer to their RDT and noted the time for reading results, the next participant should begin the next RDT.

(Otherwise, if the group were to wait until results appear for one RDT before beginning the next, this section would take about an hour.)

— 4.6 —

After each RDT is completed, group members should discuss which steps the health worker performing the test completed correctly and which steps incorrectly.

— 4.7 —

You, as the trainer, should rotate among all the groups, observe each participant's technique, and provide coaching and assistance as necessary.

➤ *You may want to ask several health workers with RDT experience to help you provide assistance and coaching. If you have a sufficient number of helpers, you can assign each helper to one or two groups.*

➤ *Don't forget to provide encouragement and reassurance for health workers nervous about performing a finger-prick for the first time.*

— 4.8 —

Once each health worker has completed 2 RDTs, bring all participants back together in 1 group.

- Ask participants to talk about their experiences carrying out the RDTs.
- Ask what steps they found easy.
- Ask what steps they found difficult.
- Once participants have had a chance to discuss and ask questions, point out any important issues you observed during the practice session (e.g., people seemed to have trouble collecting the blood from the finger, or everyone remembered to dispose of their lancet in the sharps box, etc.).
- If you had helpers, ask what they observed.

➤ *This orientation is **competency based:** each participant must **demonstrate** that he or she can perform the RDT correctly and safely, and interpret results correctly, before being allowed to use RDTs in their own village or catchment area. Participants who have not demonstrated correct and safe procedure should keep practicing until they do.*

➤ *Any participant suffering a lancet-prick injury should be started on HIV/AIIDS prophylaxis.*

Section 5 Read test results *(35 minutes)*

Learning Objective:

Participants will gain proficiency at correctly interpreting the different possible RDT outcomes.

Activities to cover:

a. Explain the three possible test results (G6PD normal, G6PDdeficient, invalid) using the job aid as a guide.

b. Ask participants to interpret the results of their own tests. Check to make sure they have interpreted correctly.

Specific steps:

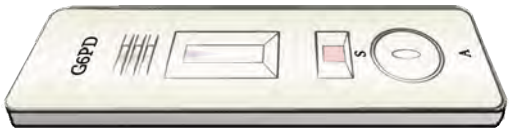
— 5.1 —

Start by going back over the pictures you have drawn on the easel pad. Instruct participants to follow along by looking at the pictures of different test results at the bottom of their job aids.

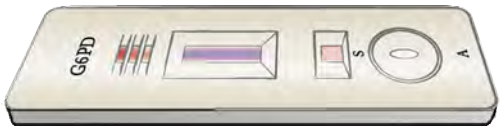
5.1.1 Review different possible results.

- The test is read as **G6PD deficient** if the background is white. This means the patient does have G6PD deficiency.

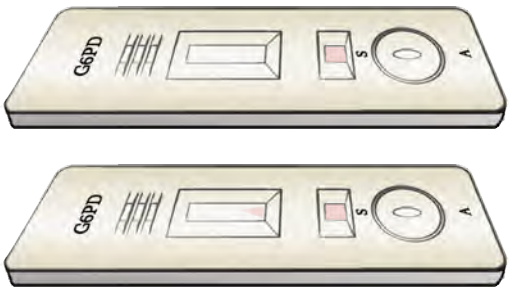
- The test is read as G6PD deficient even if a very faint purple colour appears in the results window.



- The test is read as **G6PD normal** if a distinct purple colour appears in the results window. This means the patient does not have G6PD deficiency.



- Absence of blood migration or incomplete blood migration in the window means the test is damaged. Results are **Invalid**.



5.1.2 Ask participants what to do in case of an invalid result (Answer: repeat the test using a new RDT).

— 5.2 —

Ask participants to write down on a piece of paper the results from the RDTs they completed in the last section and the immediate action they would take as a result.

If participants have brought their health worker register to the orientation session, they should write the results in the register just as they would write results for a regular patient. Some countries may have standard cards or registries to record G6PD status. Trainees should be instructed on how to complete these forms and what record to provide to patients.

Rotate among all the groups and check to make sure that all participants have recorded their results correctly. If you have assistants, they may help you with the checking.

During testing, poor vision can make it difficult for some health workers to interpret results correctly, especially in the case of a faint purple colour. As you circulate, try to determine if this is a problem for any of your participants.

Even with excellent vision, faint colour change can be difficult to detect in dim light. If they are like most health workers, some of your participants are likely to be working in dim light (e.g., trying to determine a test result at night in a setting with no electric lighting). Remind participants that they will need to have sufficient light at night: a strong flashlight or kerosene pressure lantern bright enough to illuminate even a faint colour change.

Successful completion of Section 5 concludes this orientation.

Frequently asked questions

During the testing period for this orientation, participants asked the following questions:

Q: Can I use an RDT on more than one person?

A: No. Each cassette may be used only once. You need a new, unopened cassette for each patient. If you get an invalid result from one cassette, you need a new, unopened cassette to retest the same patient.

Q: Is it possible to get a G6PD deficient result if the patient doesn't really have G6PD deficiency?

A: Yes. If the patient has an abnormally low haematocrit level a false deficient result may occur.

Q: Is it possible to get a G6PD normal result when the patient really does have G6PD deficiency?

A: Yes. If the patient has an abnormally high haematocrit level a false normal result may occur. Additionally, RDTs can only distinguish patients with < or > 30 % of normal G6PD activity. Therefore, they not distinguish homozygous females with normal G6PD activity (> 80%) from heterozygous women with intermediate G6PD activity (31-80%). This could result in some heterozygous females with intermediate activity being categorized as normal. Follow national guidelines for radical cure of *P. vivax* in females.

Q: If I don't have buffer, can I use plain water or some other liquid to perform an RDT?

A: No. Only the buffer packaged with the RDTs should be used.

Q: If I don't have an alcohol swab, can I use cotton wool and sterilizing alcohol (spirit) to clean the patient's finger?

A: Yes, you can use cotton wool and sterilizing alcohol instead of an alcohol swab. As with the swab, you should not blow on the finger or dry it with anything.

Q: What if I don't have lancets but have all the other materials I need? can I still do the test?

A: If you do not have lancets, you can use a sterile hypodermic needle from an unopened package to do the finger-prick. Once you have used the needle, you must discard it in your sharps box just as you would a lancet. The essential thing is that the instrument MUST always be sterile and unused.

Q: If I don't have a lancet, can I use a new sewing needle to do the finger prick?

A: No. A sewing needle would not be sterile. A sterile lancet or a hypodermic needle from a sterile package are the only things you can use to do a finger prick. Also, you must use a lancet only ***once and discard it immediately after the finger prick, even before collecting the blood. Never*** use a lancet or hypodermic needle on more than one patient.

Q: Why do I have to remove my gloves immediately after adding buffer to the RDT?

A: The gloves may have become contaminated with blood while you were doing the test. If you touch something while waiting for the test results, you could contaminate it with the gloves. If you examine other patients while you are waiting, you cannot keep wearing the gloves you have used with one patient while you attend another. Even if you are just sitting and waiting, blood on the gloves may contaminate other objects (such as pens) and eventually be ingested. Use a new pair of gloves with each patient.

Q: Is it OK to throw gloves, wrappers, and other waste material in the sharps box?

A: No. If you dispose of gloves and other non-sharps in the sharps box, it will fill up very quickly. Once it is full, you won't have an appropriate place to discard sharps. You may discard the pipette in the sharps box because it is small and heavily contaminated with blood. Other than the blood-transfer device, the lancet is the only thing you should throw in the sharps box. Other waste materials can be burned or buried in a rubbish pit. However, these other materials should be considered contaminated with blood and kept away from children and animals. They should be handled and disposed of according to national policy.

Q: Do I need to write down the time when the patient arrives?

A: No. After you add the buffer to the cassette, you should write the time the buffer was added and the time the results will be ready (for example, 10 minutes, depending on the RDT, after the buffer was added) on the test cassette. This is especially important if you are testing several patients in a row. You do not need to record the patient's arrival time.

Q: Why do you write down the time after adding the buffer and not after adding the blood?

A: The test only begins to work after you add buffer. Thus, you need to wait the correct time after adding the buffer, not after adding the blood. However, buffer should be added to the RDT immediately after adding the blood, before the blood dries.

Q: How long will results remain visible?

A: The RDT should be read as close as possible to the time stated in the instructions (e.g. 10 minutes). Reading the test too soon or after the recommended reading time can lead to inaccurate interpretation of the results.

Q: Can I puncture the patient's finger anywhere, as long as I choose the 4th finger?

A: The best place to puncture is on the side of the finger tip, but not too close to the nail, or midline. Puncturing in other places causes more pain to the patient and may increase the risk of infection. However, if there is some reason why you cannot puncture the 4th finger, you may puncture the middle or index finger instead.

Q: Can you throw out the lancet anywhere after using it?

A: No. You should discard the lancet ONLY in an approved sharps box. Discarding the lancet in an approved sharps box reduces the risk of someone being injured or infected by accidentally stabbing themselves.

Q: What do the markings on the RDT mean?

Trainer: before responding, you should check the following information to be sure it is accurate for the RDT you will be using.

A: Example answer:

The square hole with the letter 'S' is where you add the blood, and the large round hole with the letter 'A is where you add the buffer. To the left is a rectangular space called the results window, where you read the results.

References

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6. Espino FE, Bibit JA, Sornillo JB, Tan A, von Seidlein L, Ley B. Comparison of Three Screening Test Kits for G6PD Enzyme Deficiency: Implications for Its Use in the Radical Cure of Vivax Malaria in Remote and Resource-Poor Areas in the Philippines. PLoS One 2016; 11(2):e0148172.
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8. Access Bio, CarestartTM G6PD: Rapid Screening test for G6PD deficiency in human blood, Instructions for use.

National Malaria Control Service Guidelines on malaria management (essential).

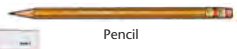
Product instructions provided by manufacturer. (Always review manufacturer's instructions before beginning your training, and clarify these instructions whenever necessary.) Testing for G6PD deficiency for safe use of primaquine in radical cure of *P. vivax* and *P. ovale* malaria. Policy Brief. 2016. WHO/HTM/GMP/2016.9

How To Do the Rapid Test to Detect G6PD Deficiency

Collect:

a. **NEW unopened** test package
b. **NEW unopened** alcohol swab
c. **NEW unopened** lancet
d. **NEW** pair of disposable gloves
e. Buffer
f. Sterile gauze or cotton wool
g. Timer
h. Sharps box
i. Pencil or pen


Disposable gloves


Pencil

Alcohol swab

Timer


Buffer

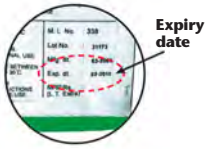
Test package


Lancet


Sterile gauze

READ THESE INSTRUCTIONS CAREFULLY BEFORE YOU BEGIN.

1. Check the expiry date on the test package.



2. Put on the gloves. Use new gloves for each patient.

3. Open the package and remove:


a. Test

b. Desiccant sachet (verify colour indicator, if applicable)

4. Write the patient's name on the test.

5. Open the alcohol swab. Grasp the 4th finger on the patient's left hand. Clean the finger with the alcohol swab. Allow the finger to dry before pricking.

6. Open the lancet. Do not allow the tip of the lancet to touch anything before pricking the patient's finger. Prick patient's finger to get a drop of blood.

7. Discard the lancet in the sharps box immediately after pricking the finger. Do not set the lancet down before discarding it.

8. Wipe away the first drop of blood with a sterile gauze or cotton wool, then discard in the infectious waste bin (or sharps box if this is not available). Gently squeeze the finger to allow a drop of blood to form. Use a pipette to collect the drop of blood. Gently hold the pipette (indicated by the blue arrow) and touch the drop of blood.

9. Use the pipette to put the correct volume of blood into the sample hole marked "S." Cover and press the hole on the pipette to release the blood.

10. Discard the pipette in the sharps box or the infectious waste bin.

11. Add two drops of buffer into the round hole marked **"A."**

12. Wait **10 minutes** after adding buffer.

13. Read test results.
(**NOTE: Do Not** read the test before **10 minutes** after adding the buffer. You may get **FALSE** results.)

15. How to read the test results:

G6PD DEFICIENT

No colour change, or a very faint purple colour means the patient **HAS** G6PD deficiency.

G6PD NORMAL

A distinct purple colour means the patient **DOES NOT** have G6PD deficiency.

INVALID RESULT

Invalid results are either where there is no blood flow or incomplete blood flow across the results window at the time of reading.

If you get an INVALID result, repeat the test using a **NEW unopened** test package and a **NEW unopened** lancet.

16. Dispose of the gloves and alcohol swab in the infectious waste container. If not available, then put them, along with desiccant and packaging in a general waste container.

17. Record the test results in your health worker register. Dispose of cassette in non-sharps waste container.

NOTE: Each test can be used **ONLY ONE TIME**. Do not try to use the test more than once.

Adapted from malaria RDT generic job aids produced by World Health Organization, United States Agency for International Development, TDR, Foundation for Innovative New Diagnostics, and URC

G6PD RDT Quiz 1

1

2

3

4

5

6

7

8

9

10

G6PD RDT Quiz 2

1

2

3

4

5

6

7

8

9

10

G6PD RDT Quiz 3

1

2

3

4

5

6

7

8

9

10

Answer keys to quizzes

Quiz #1
G6PD RDT

	G6PD Deficient	G6PD Normal	Invalid
1.		✔	
2.	✔		
3.		✔	
4.			✔
5.		✔	
6.		✔	
7.	✔		
8.		✔	
9.	✔		
10.			✔

Sample test #1

1. Normal
2. Deficient
3. Normal
4. Invalid
5. Normal
6. Normal
7. Deficient
8. Normal
9. Deficient
10. Invalid

Quiz #2
G6PD RDT

	G6PD Deficient	G6PD Normal	Invalid
1.	✔		
2.		✔	
3.	✔		
4.		✔	
5.			✔
6.		✔	
7.	✔		
8.			✔
9.		✔	
10.	✔		

Sample test #1

1. Deficient
2. Normal
3. Deficient
4. Normal
5. Invalid
6. Normal
7. Deficient
8. Invalid
9. Normal
10. Deficient

Quiz #3
G6PD RDT

	G6PD Deficient	G6PD Normal	Invalid
1.		✔	
2.	✔		
3.		✔	
4.			✔
5.			✔
6.		✔	
7.	✔		
8.		✔	
9.	✔		
10.		✔	

Sample test #1

1. Normal
2. Deficient
3. Normal
4. Invalid
5. Invalid
6. Normal
7. Deficient
8. Normal
9. Deficient
10. Normal

Blank answer sheets for quizzes

NAME

DATE

TEST

	G6PD Deficient	G6PD Normal	Invalid
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			

TEST

	G6PD Deficient	G6PD Normal	Invalid
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			

TEST

	G6PD Deficient	G6PD Normal	Invalid
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			

TEST

	G6PD Deficient	G6PD Normal	Invalid
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			

Notes