



Workshop on G6PD testing using a G6PD Biosensor

Chakaria, Bangladesh, 9th & 10th October 2019

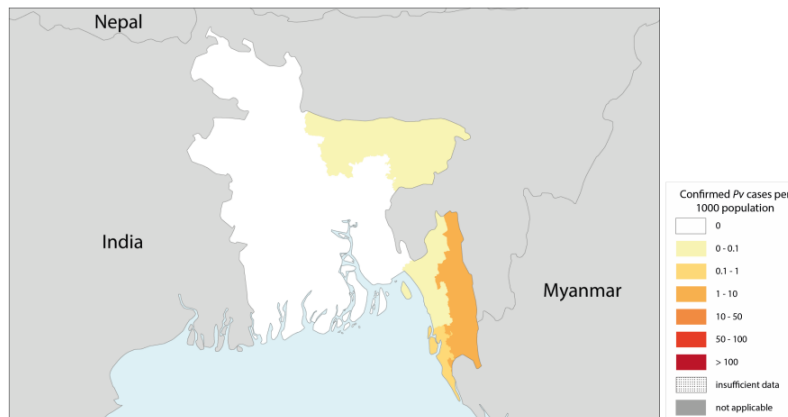
Adapted from a training presentation developed by PATH (version August 2019) for the use in a training workshops on the STANDARD™ G6PD test, by FIND in collaboration with the Menzies School of Health Research (Australia), the icddr.b (Bangladesh) and the University of Maastricht (Netherlands). This document may be freely reviewed, quoted, reproduced or translated, in part or in full, provided the source is acknowledged.

- 1 Workshop objectives and agenda
- 2 *P. vivax* radical cure and G6PD
- 3 G6PD testing
- 4 SD Biosensor STANDARD
G6PD test

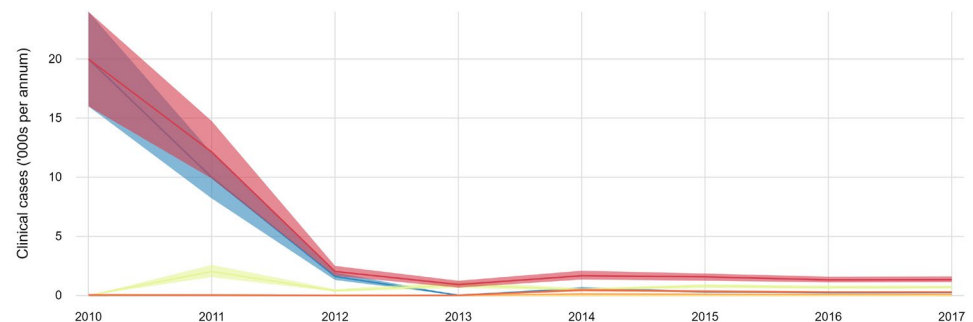
Background – why are we having this workshop?

- Major efforts are being made to control and eliminate malaria – globally and in Bangladesh
- Greatest malaria burden in Bangladesh is here in Chittagong division
- *P. vivax* malaria is a concern because of relapses after standard treatment: harder to treat and eliminate than *P. falciparum*

P. vivax in Bangladesh



CLINICAL CASES ('000s PER ANNUM) BY ADMINISTRATIVE UNIT



Background – why are we having this workshop?

OBJECTIVES OF THE WORKSHOP:

- Importance of *P. vivax* radical cure
- Discuss the role of G6PD testing
- Introduce a point-of-care quantitative diagnostic screening tool: the STANDARD G6PD Biosensor

- Practical training session with the diagnostic
- Collect participants' opinions and feedback (Focus Group Discussion)
- Evaluate the training efficiency

AGENDA:

9:00 am – 9:30 am	Registration	
9:30 am – 9:40 am	Opening remarks by NMEP representative	
9:40 am – 9:50 am	Introduction: everyone presents themselves	
9:50 am – 10:45 am	Objectives and Introduction to the G6PD point of care test	
10:45 am – 11:15 am	Tea Break	
11:15 am – 12:55 pm	Practical training with SD Biosensor G6PD test	
12:55 pm – 13:00 pm	Split into two teams of 9 (groups A and B)	
1:00 pm – 2:00 pm	Lunch and prayer break	
	Group A (9 participants)	Group B (9 participants)
2:00 pm – 3:00 pm	Practical session with observations	Focus Group Discussion
3:00 pm – 4:00 pm	Focus Group Discussion	Practical session with observations
4:00 pm – 4:15 pm	Certificate distribution and tea	

IMPORTANT NOTE:



- G6PD testing is not currently required by the national guidelines in Bangladesh. NMEP have no immediate plans to implement G6PD testing in Bangladesh.
- This workshop is a research study focused on the G6PD Biosensor diagnostic and will not cover clinical case management
- This workshop is a research study to see:
 - How well you think the test is adapted to your work settings
 - How well the training session prepares you for using the test
 - What suggestions you have about using this test in routine care
- This will help to adapt training tools and pave the way for efficiently implementing G6PD testing... not only in Bangladesh but also in other countries!

ANOTHER IMPORTANT NOTE



- **This presentation concerns *P. vivax* treatment, not *P. falciparum*!**
- Single-dose primaquine is recommended for *P. falciparum* patients in Bangladesh.
- This low-dose of primaquine has very low risks.
- WHO guidelines indicate that G6PD testing is not needed for this.

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P. vivax malaria is complicated due to relapse



Initial *Anopheles* bite & *P. vivax* infection

Relapses
Multiple relapses:
approx. every 2
months



**Illness, anaemia,
possible complications
including death**



No test available to detect
P. vivax hypnozoites
(dormant liver forms)



**Continued transmission
Obstacle to malaria
elimination**

**Important to stop the
relapses!**

What drugs do you normally use to treat *P. vivax* malaria?

Blood Stage Treatment

Chloroquine (x 3 days)



Stop malaria clinical symptoms



Liver Stage Treatment

**Primaquine
(0.25 mg/kg x 14 days)**



Prevent relapses

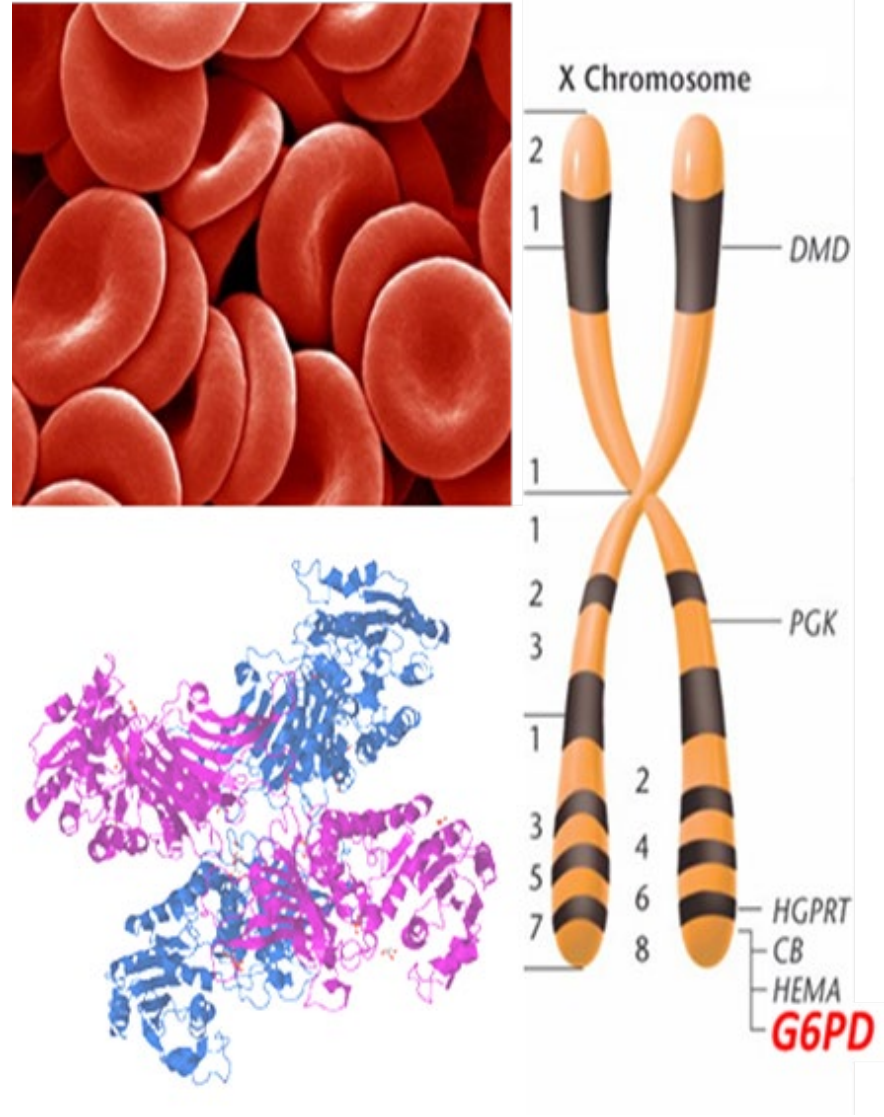
Are there any contra-indications from using CQ + PQ?

- **PQ is NOT given to:**
 - Babies < 6 months old
 - Pregnant women
 - Breastfeeding women

PQ, if given without medical follow-up or access to rapid care, can harm patients with G6PD deficiency

▶ So: what is G6PD deficiency?

Overview: What do we know about G6PD?

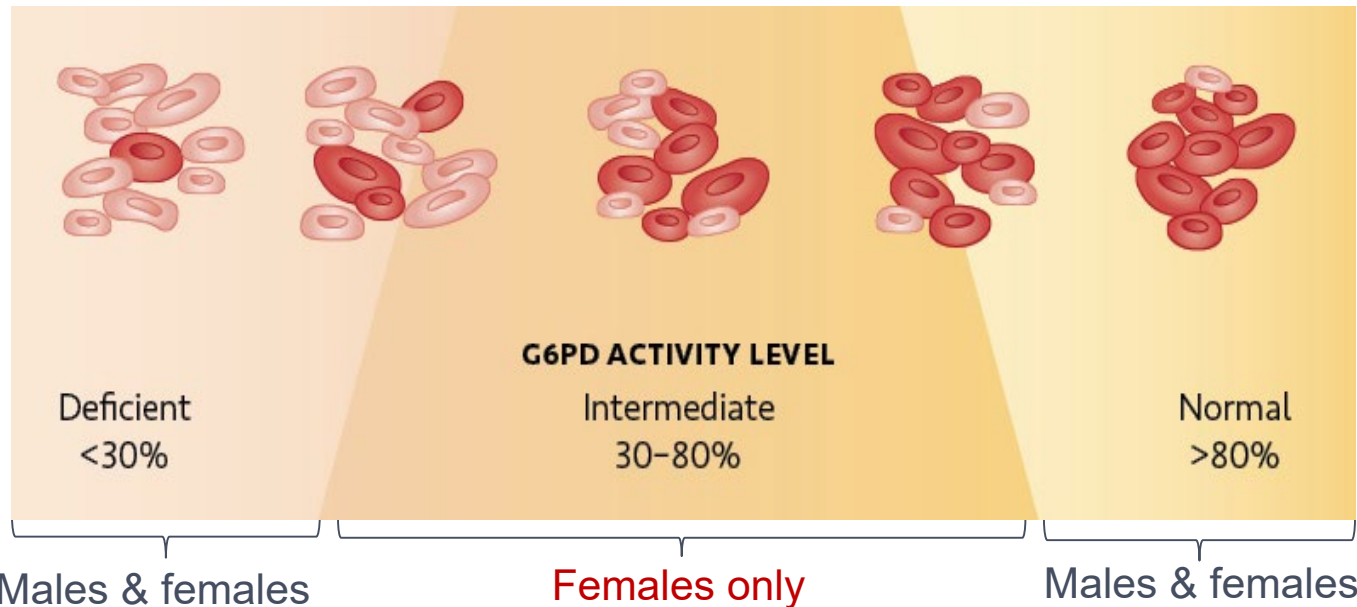


What is G6PD?

- Glucose-6-phosphate dehydrogenase (G6PD) is an essential enzyme that all people have in their bodies
- Different people have variable levels of G6PD activity. There are many types of G6PD variants with variable activity levels
- G6PD protects us from oxidative stresses (e.g. some foods, medications, infections etc)
- If someone has low G6PD activity, most of the time they will have no symptoms, but if they are exposed to an oxidative stress their red blood cells can break down (hemolyse)
- Acute hemolytic anemia (AHA) happens when red blood cells are destroyed faster than the body can replace them. This can lead to life-threatening anemia or kidney failure

G6PD Status

G6PD genetics mean that individuals can have a mix of red blood cells – some with low and some with high G6PD activity.



Deficient:
red blood cells
with mostly low
G6PD activity

Intermediate:
mixed blood cells,
with high and low G6PD activity



Most difficult group
to diagnose

Normal:
red blood cells
with mostly high
G6PD activity

G6PD deficiency in Bangladesh

- Shafiul and Ben (workshop organisers) have done recent studies of G6PD in Chittagong Division
 - 9% deficient (<30% of normal levels)
 - 22% were deficient/intermediate (<70% normal levels)
- G6PD deficiency is quite common in Bangladesh, and there are some severe types
 - e.g. G6PD^{Mediterranean}: these individuals cannot tolerate primaquine
=> important to test
- Important to collect up more data to better understand the risks for *P. vivax* patients in Bangladesh

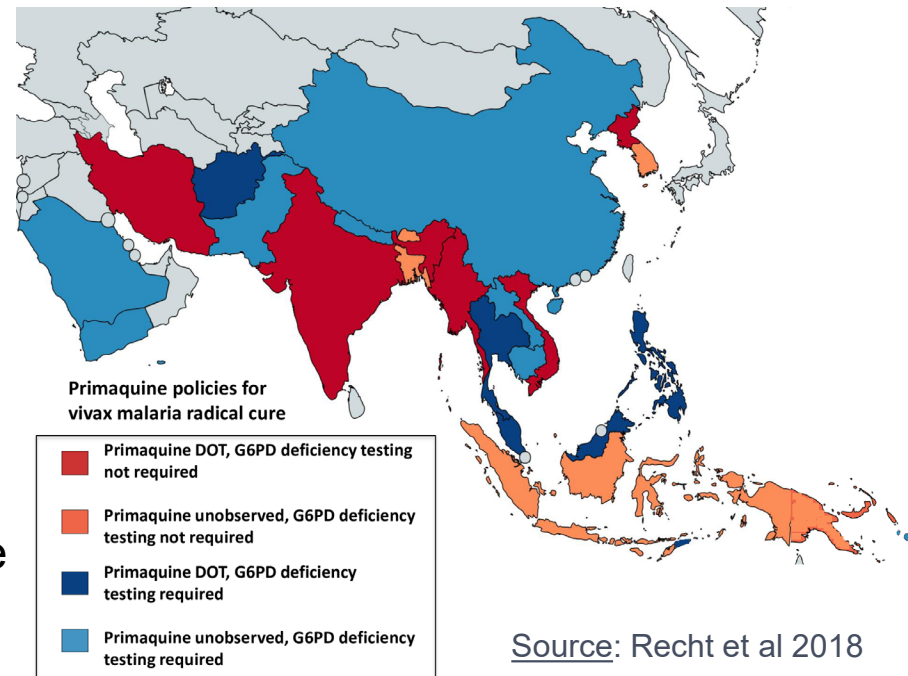
P. vivax treatment guidelines by G6PD status

WHO guidelines: if possible, G6PD status should guide who can receive primaquine treatment

Current national policies vary – see map:

Sometimes health professionals do not want to give primaquine

Having easier tests for G6PD will increase access to safe primaquine



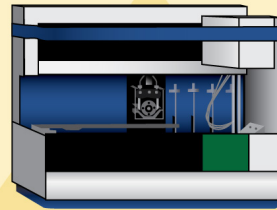
➤ This means G6PD status should ideally be known, but HOW?

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G6PD test

G6PD testing: currently available methods

High-quality, accurate, and affordable point-of-care tests for G6PD deficiency are still being developed and tested.

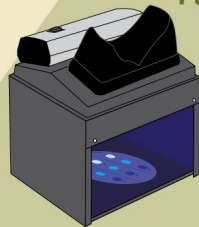
NATIONAL
REFERENCE LEVELS



High-Throughput Tests

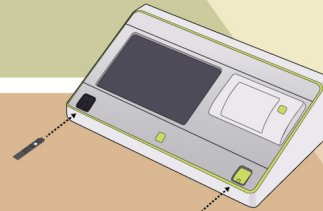
High throughput tests (e.g. spectrophotometer), far from where patients are seen.

DISTRICT AND
SUB-DISTRICT LEVELS



Fluorescent
Spot Tests

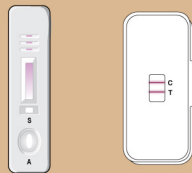
Biosensors



Quantitative methods closer to point-of-care, but still new and require thresholds.

COMMUNITY
LEVELS

Rapid Diagnostic Tests



Qualitative rapid tests ideal for point-of-care but not yet reliable enough.



G6PD testing challenges

1. Quantitative methods are needed to accurately detect females with intermediate G6PD activity
2. Most reliable and quantitative methods are not suitable for use in malaria treatment settings (point-of-care, POC)
3. Simple rapid tests are only qualitative (could miss heterozygous females) and still not reliable enough
4. Biosensors appear as the best compromise, i.e. are quantitative and can be used at the POC, however need more evidence on their performance, accuracy, ease of use, and acceptability by POC health workers

➤ This is why we need YOUR help in this workshop 😊

Acknowledgements

This work is a collaboration between:



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Australian Government

Department of Foreign Affairs and Trade

Training slides were adapted from work done by PATH and the G6PD Operations Research Community of Practice

The competency assessment questionnaire was developed by PATH.

AGENDA:

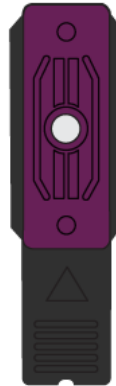
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SD Biosensor
STANDARD G6PD
Test



Test Procedure



Components



Test Device



EZI Tube
Sample Collector



Extraction Buffer

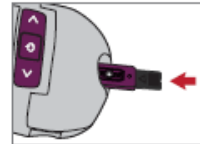


Code chip

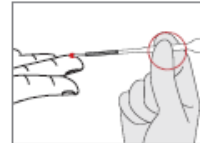


Analyzer

Test Procedure



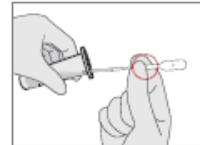
1. Insert test device into analyzer.



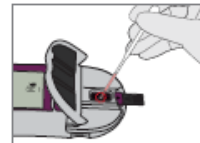
2. Collect blood.



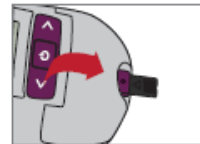
3. Mix blood and buffer 8-10x.



4. Collect mixed sample with NEW sample collector.

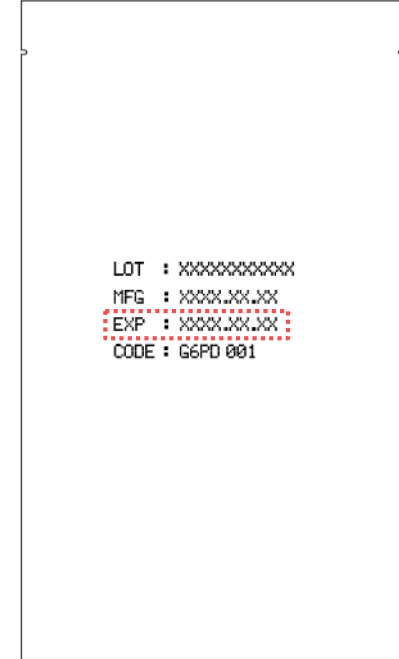
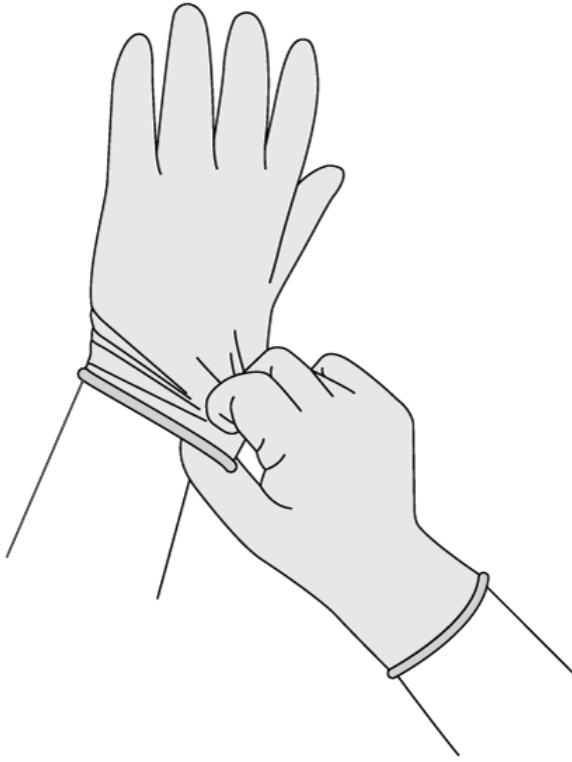


5. Apply mixed sample to test device.

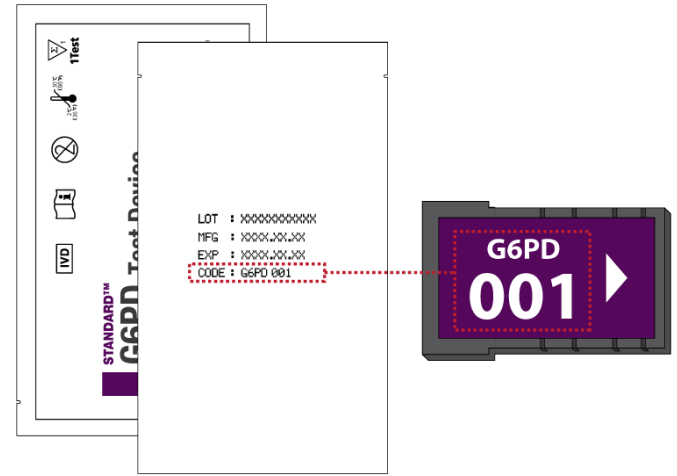


6. Close analyzer flap.

Step 1: Put on new gloves for each patient.
Prepare materials needed – check analyzer
battery and expiration of code chip.



Step 2a : Check that the code number printed on foil pouch matches the one from the code chip



Step 2b: Insert the new code chip until it snaps into place.

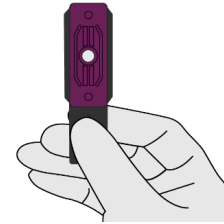
Note: Make sure the analyzer is turned off.
There is a code chip in every kit.
Replace the code chip only when you open a new kit.



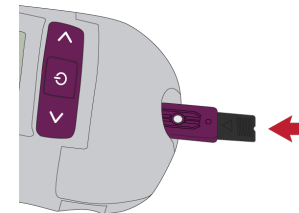
Step 3a : Open the foil pouch with test device and take a test device out.



Step 3b: Hold the test device with thumb and index finger so that the upper test device is facing upward.

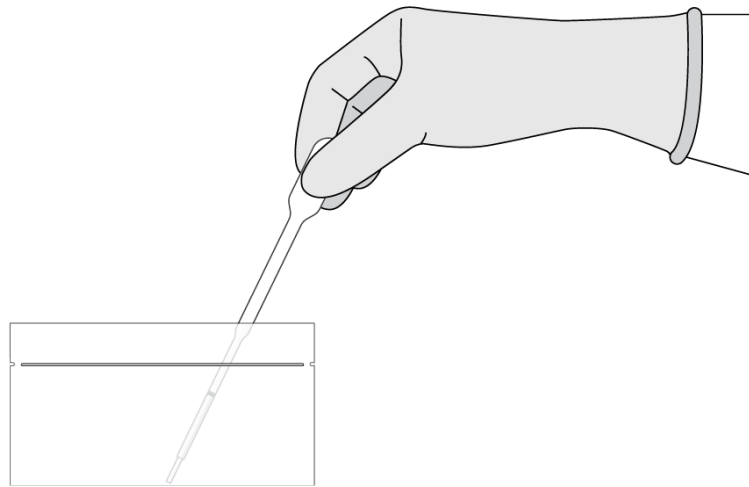


Step 3c: Insert the test device into the test device slot until it will go no further.



Step 4: Open the sample collector pouch and take out a NEW sample collector.

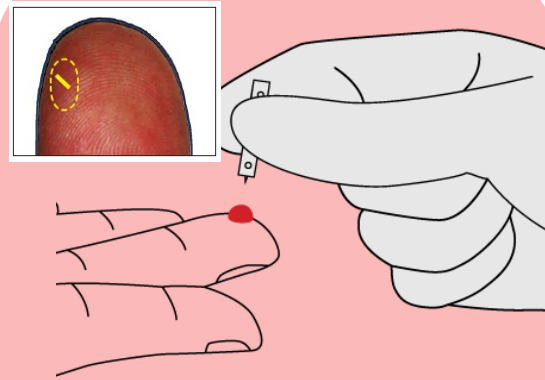
Place in a tube rack or on a clean surface



Step 5a: Finger prick to collect blood sample



1. Disinfect with alcohol swab
2. Let alcohol air dry



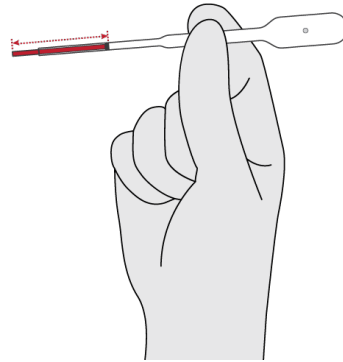
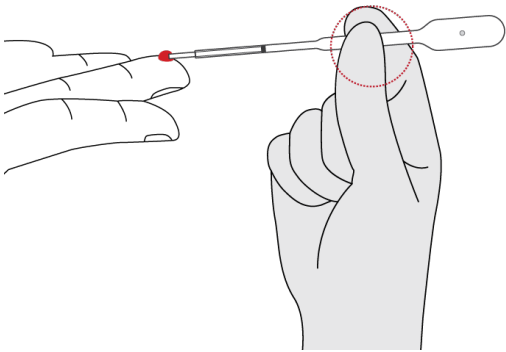
3. Prick finger:
quick firm stab on side of finger

4. Discard lancet into sharps box immediately

5. Squeeze finger by pushing towards fingertip to produce big drop of blood

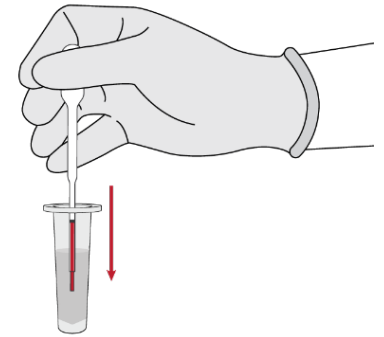
(Finger massage before pricking can help collect more blood)

Step 5b: Hold the sample collector horizontally and touch the tip of the sample collector to the blood specimen.

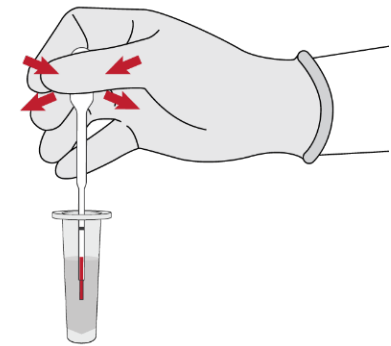


Note: Capillary action will automatically draw the specimen to the black line and stop, on its own.

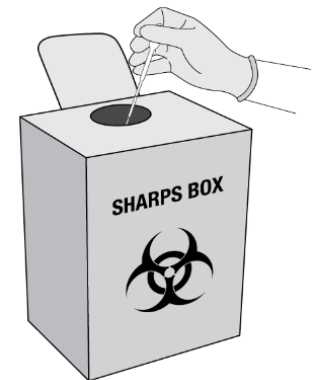
Step 6a: Place the sample collector into the extraction buffer.



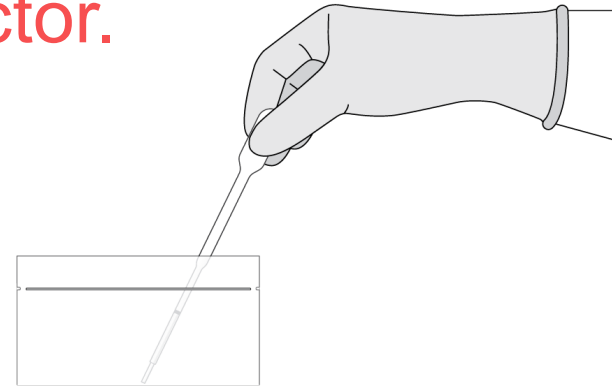
Step 6b: Mix the collected specimen with extraction buffer, pressing and releasing the sample collector 8 to 10 times.



Step 6c: Discard used sample collector in the biohazard box.



Step 7a: Open the sample collector pouch and take out a NEW sample collector.

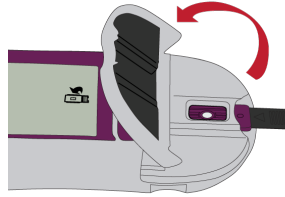


Step 7b: Hold NEW sample collector horizontally and touch the tip of the sample collector to the mixed specimen.

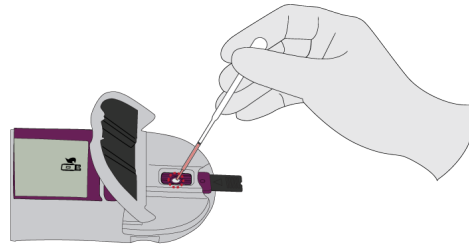


Note: Capillary action will automatically draw the specimen to the black line and stop, on its own.

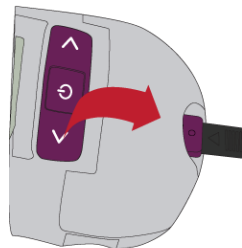
Step 8a: Open the measurement chamber flap.



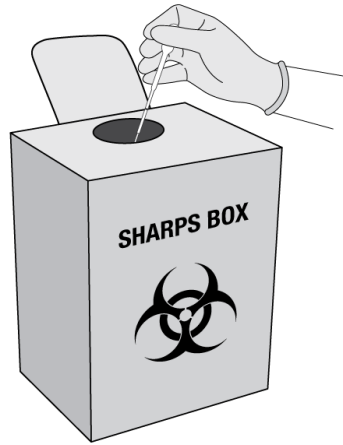
Step 8b: Apply the mixed specimen to the specimen application hole of the test device.



Step 8c: Close the measurement chamber flap **IMMEDIATELY** after applying.



Step 9a: Discard used sample collector in the sharps box.



Step 9b: Wait 2 minutes for the test result to appear on the screen.

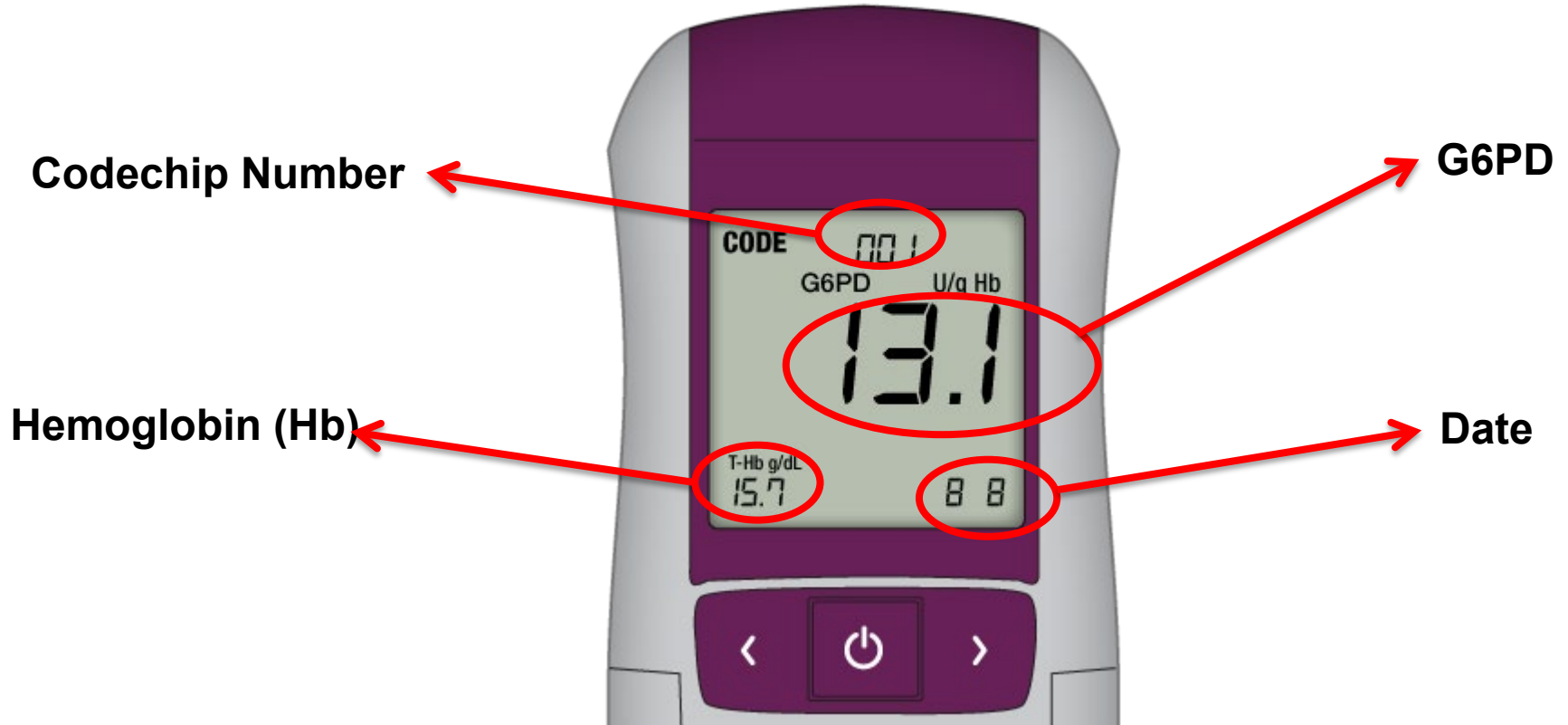


SD Biosensor G6PD STANDARD Test Video

Insert video of the test being performed

[provided in a separate file]

Results Output



Results Interpretation

The result of G6PD activity in IU/g Hb will allow the patient to be classified as G6PD normal, intermediate or deficient.

The ranges for these categories vary between populations.

Consult the local recommendations that will be provided when you receive the test.

Results Interpretation:

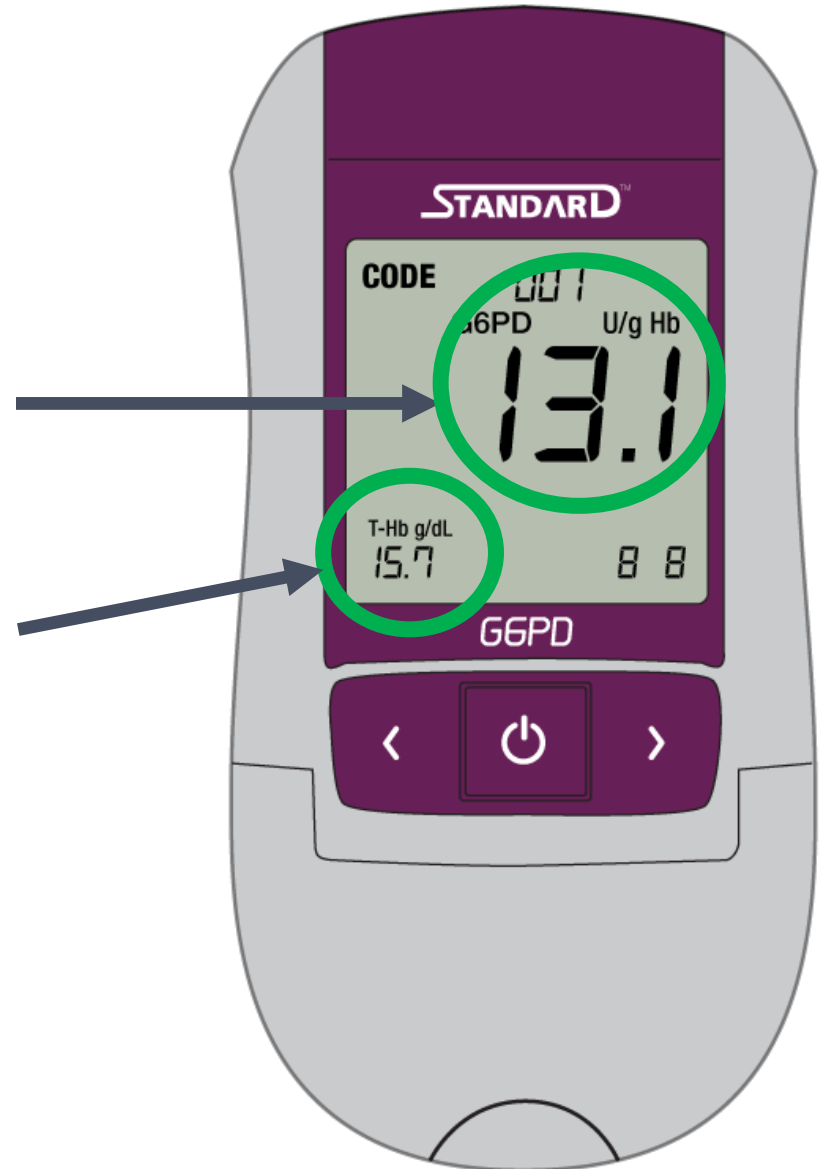
indicative thresholds for Chittagong Hill Tracts populations

G6PD Biosensor activity (IU g/Hb)	G6PD category
> 6.0	Normal (>70%)
2.6 – 6.0	Intermediate (30 – 70%)
< 2.6	Deficient (<30%)

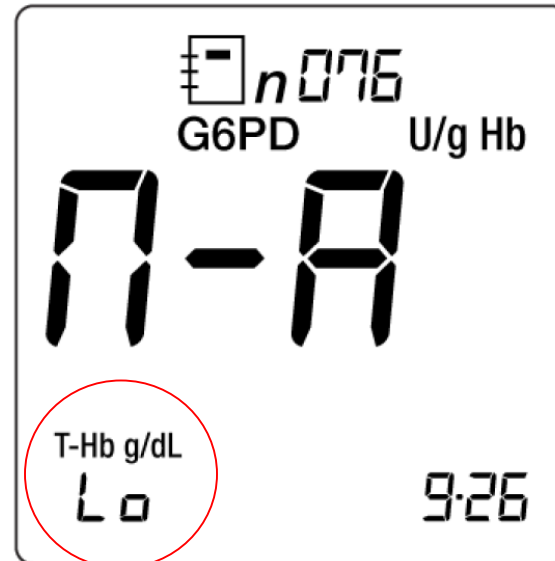
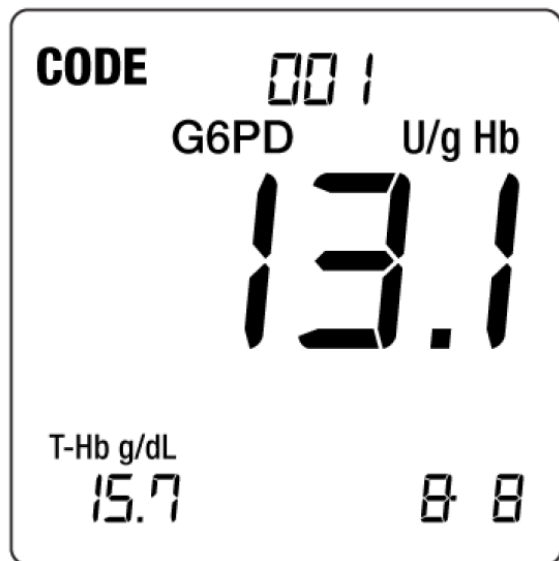
Results Interpretation

G6PD enzyme activity
(unit: U/g Hb)

Hemoglobin concentration
(unit: g/dl)

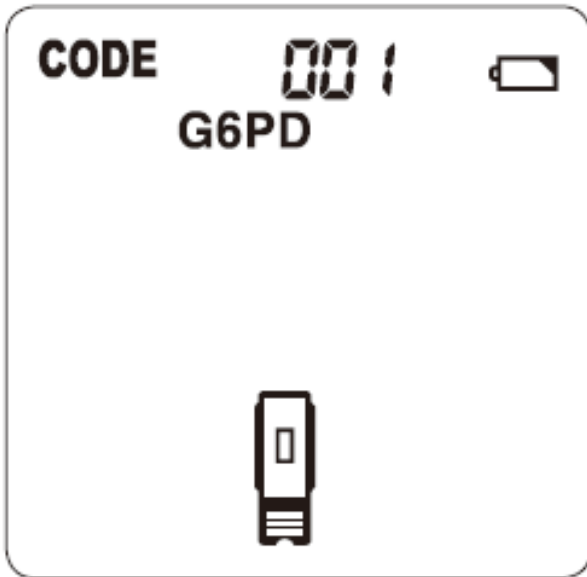


Results Interpretation



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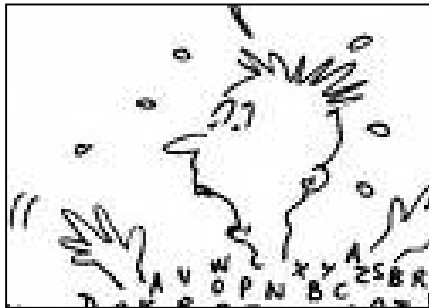
Results Interpretation



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Any questions?



PERFORMING A CHECK STRIP TEST



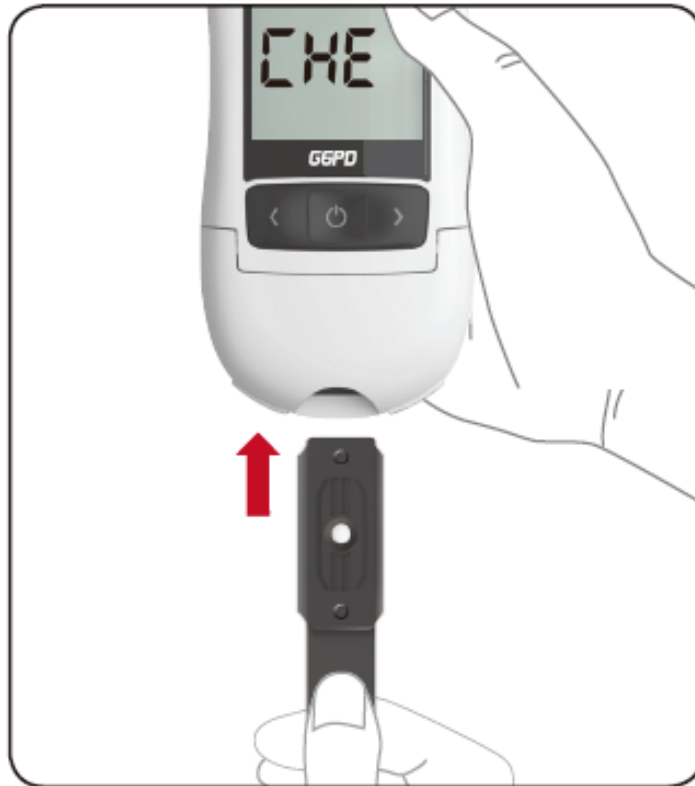
When to Use STANDARD G6PD Check Strip

- When using your analyzer for the first time.
- When opening a new test device packaging.
- Whenever your result does not agree with the test result you expected.
- When replacing the batteries or cleaning the analyzer.
- When dropping the analyzer.

How to Use a STANDARD G6PD Check Strip



1. Switch on the analyzer and press Press Left and Right button together for 3 seconds to enter the check strip test mode.



2. When the 'CHE' appears on the screen, insert a check strip.
3. Wait 10 seconds.



4. If there is any problem for the analyzer, 'EEE' error message will appear on the screen. Otherwise, 'OK' message will appear on the screen.

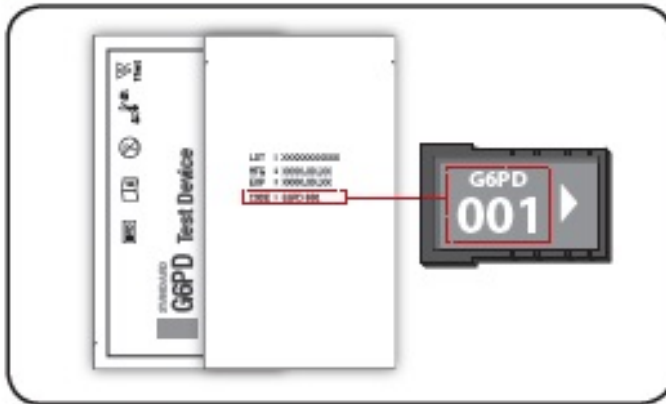
PERFORMING A CONTROL TEST



When to Use STANDARD G6PD Control Test

- When opening a new test device packaging (new lot number).
- Whenever your result does not agree with the test result you expected.

Preparing to Perform a Control Test



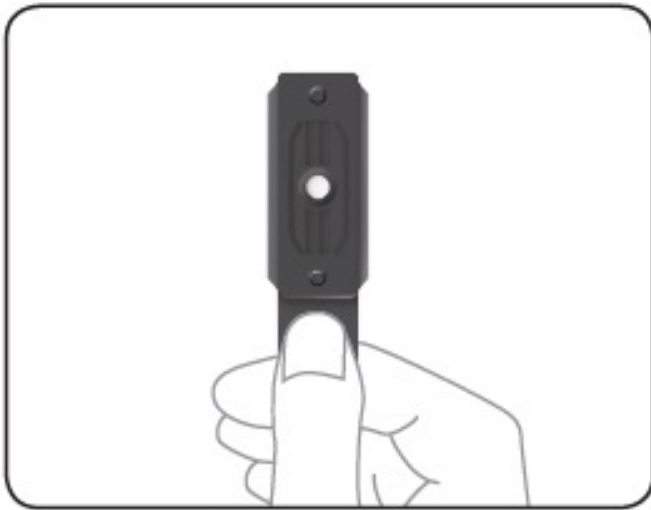
1. Check the expiry date printed on the foil pouch.
2. Check the code number on the codechip matches the code number printed on the test device pouch

Inserting the Codechip

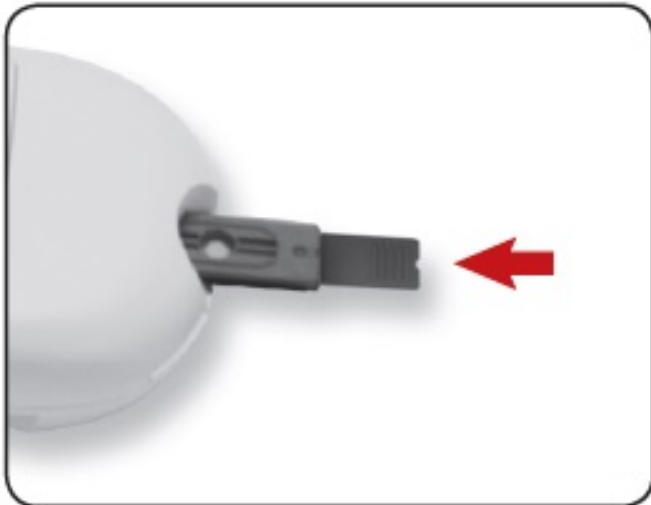


1. While changing the code chip, the analyzer must be turned off. Remove an old codechip if one is installed.
2. Insert a new codechip and turn on the Analyzer.
3. And then the Analyzer is turned on, the codechip number will appear on the screen.
4. Make sure the codechip number matches with the number on the screen

Inserting the Test Device



1. Open the foil pouch and take a test device out.
2. Hold the test device with thumb and index finger so that the upper test device is facing upwards.



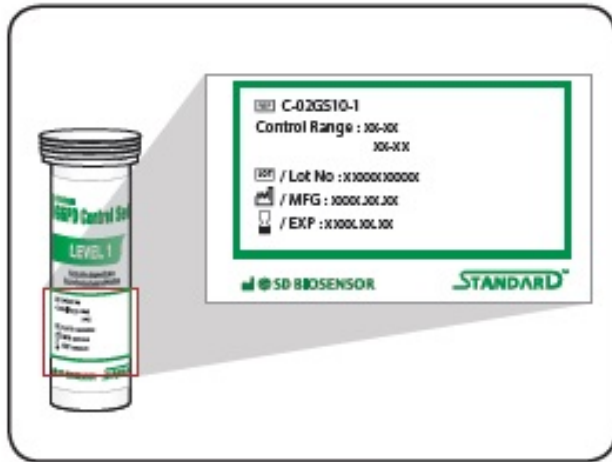
3. Insert the test device completely into the test device slot of a STANDARD G6PD Analyzer.
4. The analyzer automatically turned on when the codechip is correctly inserted.



5. The display of 'OPE' to open the Measurement Chamber Flap will appear on the screen.
6. Press the left button for 3 seconds. "C" mark will be appeared on the screen. "C" mark represents control test mode.



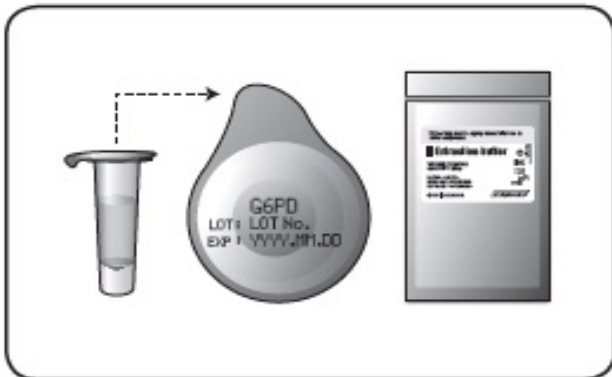
7. Open the measurement chamber flap.



1. Check the expiry date printed on the control bottle.

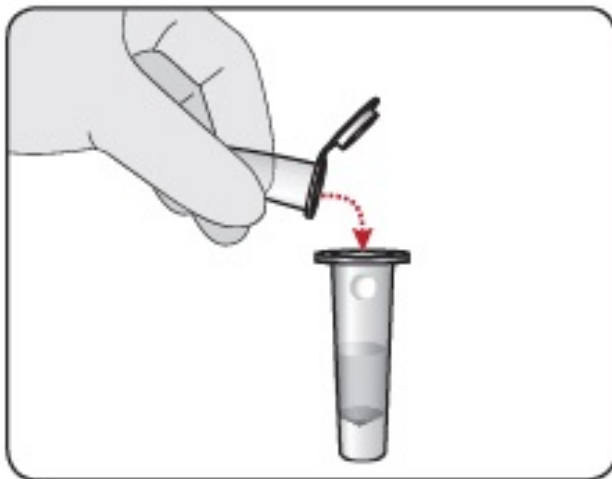


2. Open the control bottle and take a tube out of the bottle.

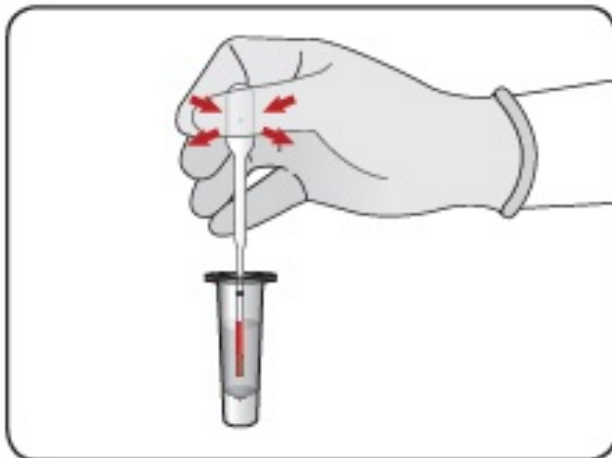


3. Open an extraction buffer pouch and take an extraction buffer out of the pouch.

※ An extraction buffer should not be used beyond the printed expiration date, EXP.



4. Put the control tablet into the extraction buffer.

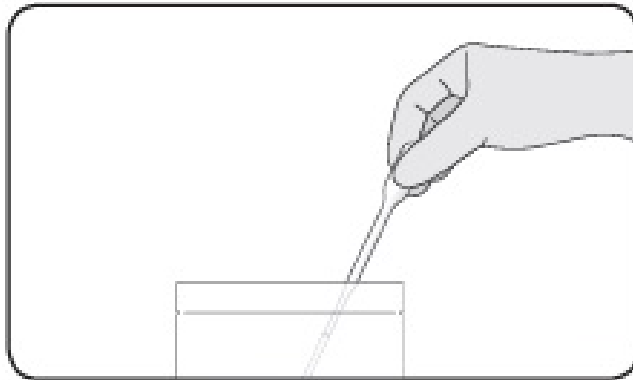


5. Mix the collected specimen with extraction buffer, pressing and releasing a new STANDARD Ezi tube+(10 μ l) 8 to 10 times with the hole closed.

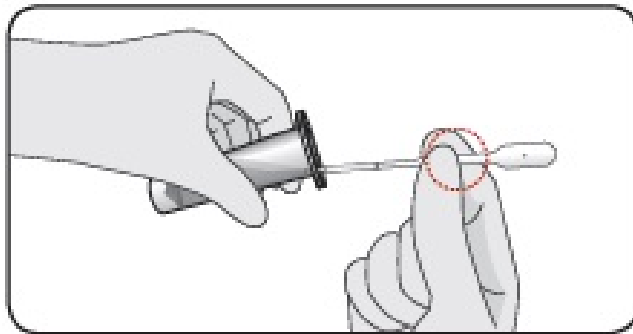
*Do not collect mixed specimen until the bubble is completely gone.



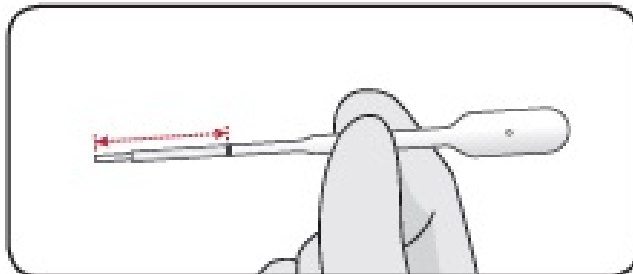
6. Discard the STANDARD Ezi tube+(10 μ l) in a biosafety box.



7. Open the STANDARD Ezi tube+(10 μ l) pouch and take an unused STANDARD Ezi tube+(10 μ l) out.



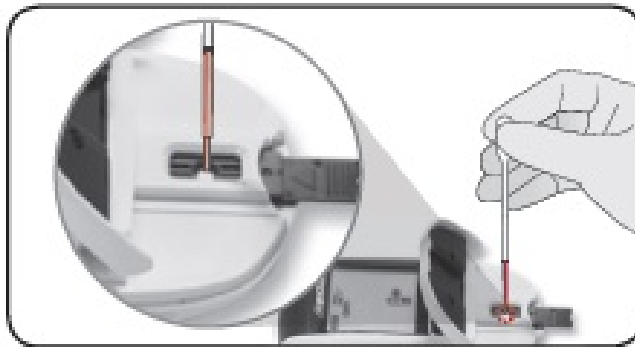
8. Hold the STANDARD Ezi tube+(10 μ l) horizontally, and touch the tip of STANDARD Ezi tube+(10 μ l) to the control material.



9. Capillary action will automatically draw the control material to the black line and stop.

*Apply the sample mixture within 1 minute after mixing the STANDARD G6PD Control sample and extraction buffer solution.

Performing a Control Test



1. Apply the control mixture to the test device.



2. The display of 'CLo' to close the measurement chamber flap will appear on the screen.



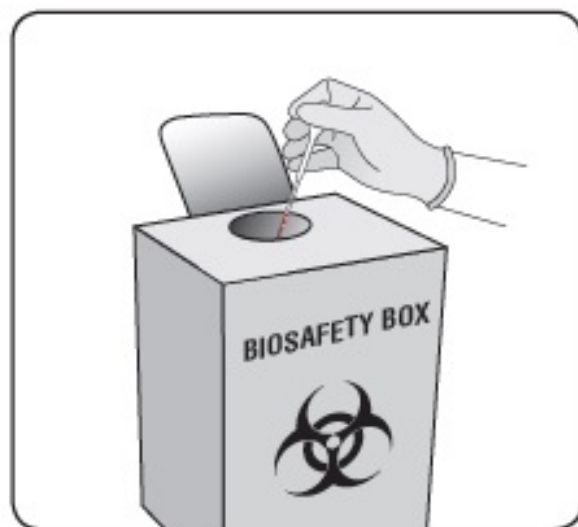
3. Close the measurement chamber flap immediately after applying.

※ Exposing the test device to direct and strong light may cause false result.



- The analyzer will automatically counts down to 2 minutes when the flap is closed.

※ Do not open the chamber flap during the measurements.



- Discard the used STANDARD Ezi tube+(10 μ l) in a biosafety box.



- After 2 minutes of reaction time, the test result will be appear on the screen.

SD Biosensor STANDARD G6PD Test IFU

Intended use

The test is intended to aid in the identification of people with G6PD deficiency, providing point-of-care access and allowing prompt treatment decisions.

Kit storage and operating temperature

The sealed pouch containing the test device may be **stored at 2°C to 30°C** (36°F to 86°F) out of direct sunlight.

Perform test at 15°C to 40°C (59°F to 104°F).

Specimen collection and preparation

Perform test using whole blood (**capillary or venous**).

If stored venous blood is kept in a refrigerator, the blood can be used for testing 24 hours after collection.

Measuring Ranges

Total hemoglobin: 4-25 g/dL

G6PD: 0-20 IU/g Hb

COMMON USER MISTAKES



Common User Mistakes

Forgot to calibrate the analyzer.

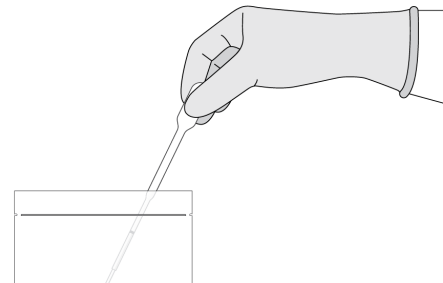
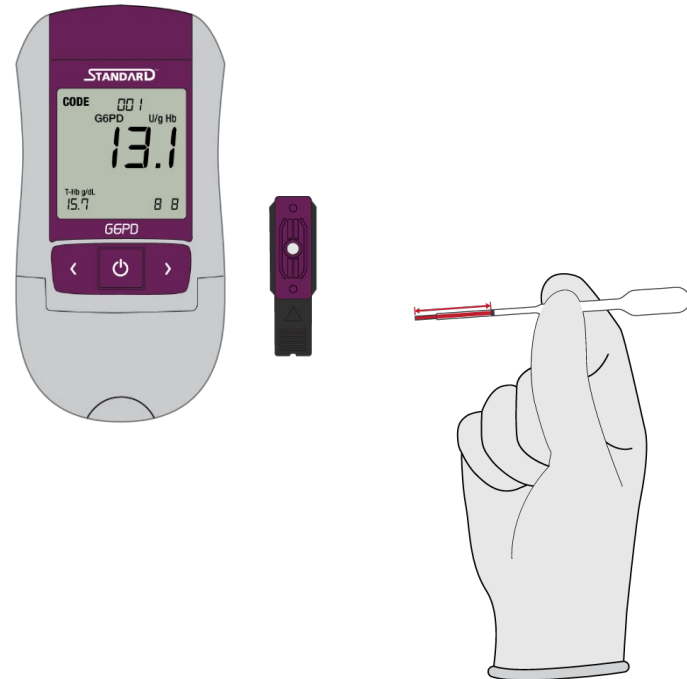
Make sure to calibrate the analyzer if using a new analyzer or a new lot of test devices, or if the analyzer has been dropped.

Did not collect a large enough sample.

Ensure you collect sufficient volume of blood and buffer. Fill the sample collector to the black line.

Used the same sample collector for collecting both blood and buffer.

Dispose of sample collector after blood collection and use NEW sample collector for buffer collection.



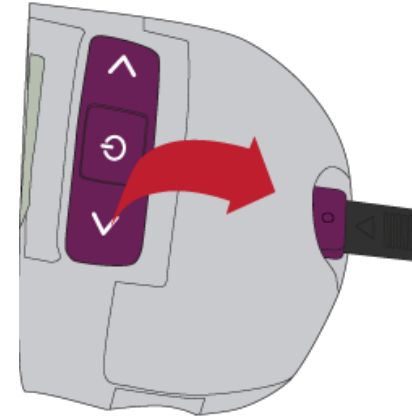
Common User Mistakes (cont.)

Waited too long to close measurement chamber and test timed out.

Close measurement chamber flap immediately after applying specimen.

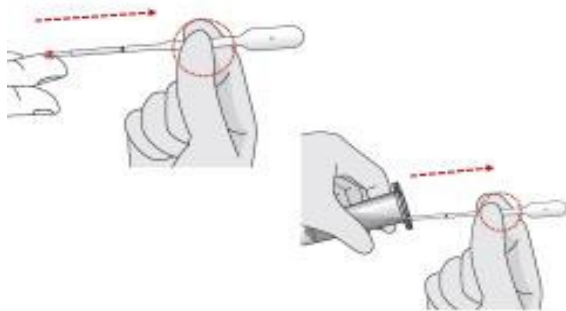
Read hemoglobin results incorrectly.

The top right 13.1 U/g Hb indicates units for G6PD measurement. The bottom left 15.7 T-Hb g/dL indicates hemoglobin measurement.

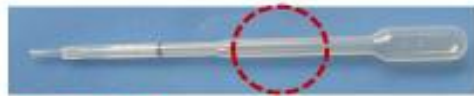
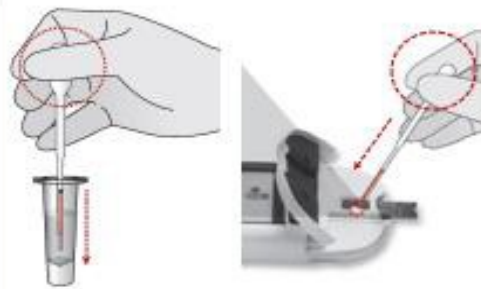


How to use the EZI tube

TO TAKE UP



TO PUSH OUT



HOLD HERE



HOLD HERE

Loading the right amount of blood and the right amount of mix is VERY important in order to have a reliable result.

While holding the EZI tube on the blood drop or on the mix, the liquid will AUTOMATICALLY fill the EZI tube up to the black line.

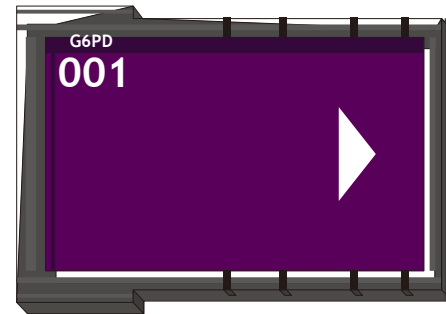
Do NOT remove the EZI tube too early. When held correctly, the EZI tube will not collect more blood than needed, as it will stop at the black line.

QUIZ



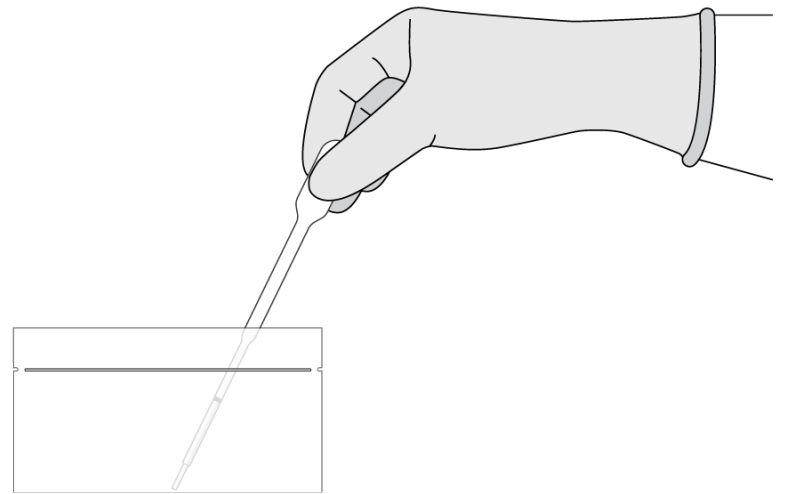
Quiz

How do you use the code chip?



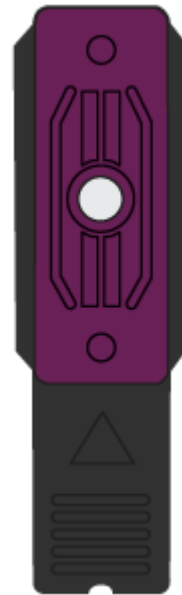
Quiz

How many sample collectors do you need to run one sample?



Quiz

Can you re-use the test device?



Quiz

What is the temperature range for test operation?



Acknowledgements

This work is a collaboration between:



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Training slides were adapted from work done by the G6PD Operations Research Community of Practice (PATH, SMRU, etc.)

The competency assessment questionnaire was developed by PATH.

AGENDA:

9:00 am – 9:30 am	Registration	
9:30 am – 9:40 am	Opening remarks by NMEP representative	
9:40 am – 9:50 am	Introduction: everyone presents themselves	
9:50 am – 10:45 am	Objectives and Introduction to the G6PD point of care test	
10:45 am – 11:15 am	Tea Break	
11:15 am – 12:55 pm	Practical training with SD Biosensor G6PD test	
12:55 pm – 13:00 pm	Split into two teams of 9 (groups A and B)	
1:00 pm – 2:00 pm	Lunch and prayer break	
	Group A (9 participants)	Group B (9 participants)
2:00 pm – 3:00 pm	Practical session with observations	Focus Group Discussion
3:00 pm – 4:00 pm	Focus Group Discussion	Practical session with observations
4:00 pm – 4:15 pm	Certificate distribution and tea	

SD Biosensor STANDARD G6PD Test IFU

Warnings

1. The STANDARD G6PD Test device should only be used with the STANDARD G6PD Analyzer.
2. Calibrate analyzer if using a new analyzer or a new lot of test devices, or the analyzer has been dropped.
3. The test device should not be used beyond the printed expiration date.
4. Make sure that the code chip and the code number printed on the pouch match.
5. The test should be performed at 15°C to 40°C (59°F to 104°F).
6. A test device is for single use only. Do not reuse.
 - Insert a test device and code chip into the test device slot and the code chip slot of the analyzer.
 - Insert a test device into the test device slot with blood application chamber facing up and toward the analyzer.
7. Insert a code chip into the code chip slot with the surface printed with the code number facing up toward the analyzer.
8. Ensure the proper specimen volume for the test device is used. The specimen volume should be 10µl.
9. Insert a test device into the analyzer gently until it will go no further.
10. Do not apply on another site except blood application area of a test device.
11. Do not ingest.
12. Discard the used test devices according to the local guidelines.
13. Extraction buffer contains Triton X-100, which can cause serious eye irritation.