National Standard Operating Procedure of Qualitative G6PD enzyme assay (Manual)

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Ministry of Health

Directorate General of Health Services and Programs

Department of Health Services Development

Diagnostic Services Development Section

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Table of Contents

Acknowledgments

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Acronyms

SOP	Standard operating procedure
G6PD	Glucose-6-phosphate dehydrogenase
GSH	Reduced glutathione
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
Hep A	Hepatitis A virus
CMV	Cytomegalovirus
UV-light	Ultraviolet light
EDTA	Ethylenediaminetetraacetic acid
ul	Microliter

1. **Purpose**

   This standard operating procedure (SOP) document will provide a guide to laboratory personnel on how to perform G6PD Fluorescent screening test, interpretation and reporting of the results.

2. **Scope**

   This document is applicable to qualified and trained technical staff in the hematology Lab in the laboratory department.

3. **Definitions**

   - **A qualitative test** is a test that determines if a specific characteristic, condition, or substance is present or absent.

4. **Procedure**

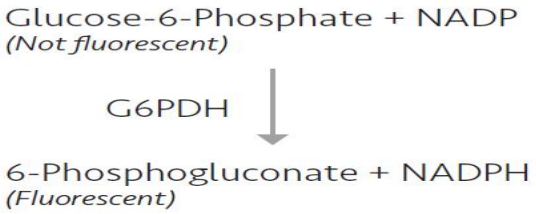
   4.1. Clinical Background

   Red cells are constantly exposed to both endogenous and exogenous oxidants, which are normally inactivated by reduced glutathione (GSH). Glucose-6-phosphate dehydrogenase (G6PD) enzyme is responsible for the production of reduced GSH through reduction of Nicotinamide Adenine Dinucleotide Phosphate (NADP). Deficiency in G6PD enzymes leave red cells vulnerable to oxidative injury and lead to hemolytic anemia. The most common cause of oxidative stress hemolysis is anemia secondary to G6PD deficiency.

   G6PD enzyme deficiency is an X-linked genetic disorder. Hemolysis occurred once patient exposed to an environmental factor that produce oxidants such as Fava bean, anti-malarial drugs (e.g. primaquine), antibiotics (e.g: cotrimoxazole, nitrofurantoin), rasburicase or certain infections (e.g: Hep A, CMV) and hena dyes.

   4.2. Principle

   G6PD catalyzes a reaction in the pentose phosphate pathway, a metabolic pathway that supplies reducing energy to cells such as erythrocytes by maintaining the level of the reduced form of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH).



In normal patient NADPH that is generated by G6PD enzyme present in the lysate of red cells fluoresces under UV-light. NADPH fluorescence is directly proportional to G6PD activity and lack of fluorescence signals indicates G6PD deficiency.

4.3. Health & safety

Universal precautions must be practice at all stages of test.

4.4. Policy

4.4.1. Hemolyzed, clotted or insufficient blood samples must not be accepted for testing.

4.4.2. Blood samples stored at 2℃ to 8℃ for up to 7 days may be used for testing.

4.4.3. This test should be done every morning during working days.

4.4.4. During weekends or other duties, tests should be run upon request in Emergency cases, such as malaria or prior to starting any medication that is known to cause oxidative hemolysis.

4.5. Sample requirement

4.5.1. Sample must be at least 2 ml EDTA whole blood to be used for testing.

4.6. Items required

4.6.1. Plastic Tubes

4.6.2. Filter Paper

4.6.3. Micropipette

4.6.4. Microtips

4.6.5. UV-light

4.6.6. Vortex Mixture

4.6.7. G6PD substrate (glucose-6-phosphate)

4.6.8. G6PD Deficient control

4.6.9. G6PD Normal control

4.6.10. Buffer.

4.7. Equipment Maintenance

Nil

4.8. Calibration/ Quality Control

Positive control and negative control must be run once in the morning daily.

4.9. Procedure Steps

4.9.1. Allow all the reagents to reach room temperature before testing.

4.9.2. Label a plastic tube with sample ID

4.9.3. Label another two plastic tubes with normal control and deficient control.

4.9.4. Add to each tube 100 µl of the substrate reagent

4.9.5. Mix the EDTA sample well and take 5 µl from whole blood

4.9.6. Wipe the blood off the outside of the pipette tip.

4.9.7. Dispense the blood into the correct tube.

4.9.8. Mix the mixture thoroughly by vortexing for 10 seconds at maximum speed.

4.9.9. Keep the tubes for 10 minutes at room temperature

4.9.10. Prepare the filter paper by labelling each square and add (5-7 µl) of the mixture to the right position in the paper.

4.9.11. Incubate the paper at 37˚c for 15 min

4.9.12. After incubation, view the filter paper under along-wave UV light.

4.9.13. Compare samples spots with controls to determine the fluorescence levels.

4.9.14. Record fluorescent intensity (normal, deficient) of each sample.

4.9.15. If the fluorescent intensity is partial/inconclusive, refer to the flowchart in Annexes section.

4.10. **Results**

4.10.1 Classify each sample based on level of fluorescence:

4.10.1.1. Normal G6PD activity (N): moderate to strong fluorescence

4.10.1.2. Deficient G6PD activity (D): very weak or no fluorescence

4.10.2 Limitation**:**

G6PD Fluorescent screening test results can be affected by many factors:

Limitation		**Trouble Shooting**
False Normal results	Blood transfusion during the last 3 months	The test must be repeated after 3 months from the last date of transfusion.
	High reticulocytes count > 5% (during acute phase)	The test must be repeated after passing the acute phase and reticulocyte count normal.
	High White blood cell count $>23\times10^{3}$\ µl	Wash the sample 3 times with normal saline (leukodepletion) and then run the test.
False Deficient results	Low hemoglobin level < 5 g\dl	Add double amount of the blood = 10µl

5. Responsibility

5.1 Laboratory Program committee:

- Ensure that document is approved by ministry of Health and distributed to all institutions

5.2 Task force creating the document:

- Ensure that the document is in accordance with Ministry of Health guidelines and is evidence based
- Ensure that the document is sent to the relevant individuals in different health institutions for review
- Ensure that the document is modified after receiving relevant feedback from reviewers
- Ensure that the document is updated where appropriate

5.3 Laboratory Head/ supervisor

- Review the document and provide feedback where appropriate
- Ensure that local standard operating procedures are in line with document
- Ensure that all staff are trained and adhere to the document

5.4 Technical staff

- Be familiar with this document and adhere to it where applicable

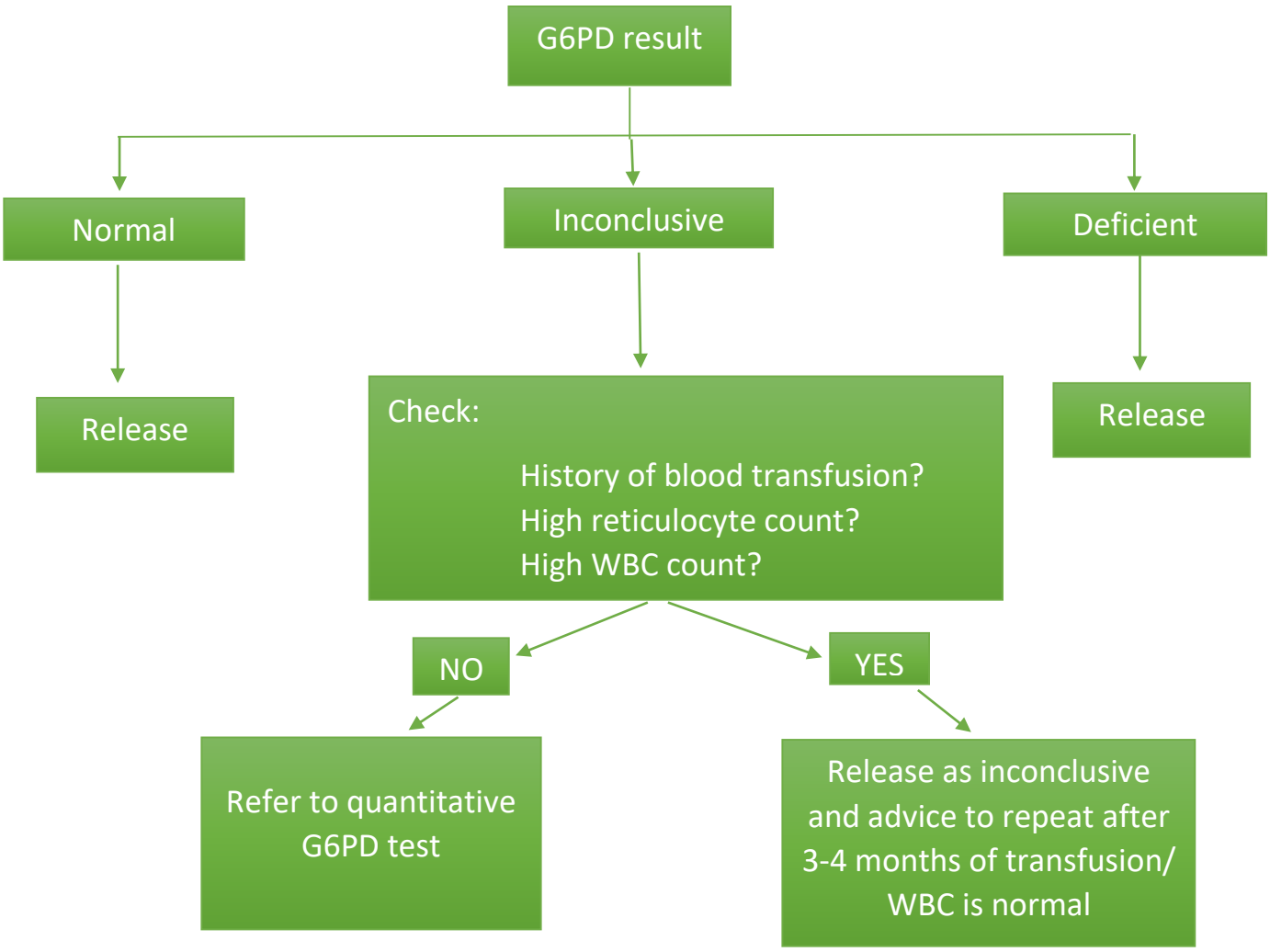
6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	August 2031

7. References

1. Hoffbrand V.A., Moss P.A.H. Hoffbrand's Essential Haematology-7th Edition. 2016.
2. Manufacturer procedural leaflet.

8. Annexes


G6PD result
Normal
Inconclusive
Deficient
Release
Check:
History of blood transfusion?
High reticulocyte count?
High WBC count?
Release
NO
YES
Refer to quantitative G6PD test
Release as inconclusive and advice to repeat after 3-4 months of transfusion/ WBC is normal