National Policy and Procedure of Quantitative G6PD Enzyme Assay (Automated)

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Acronyms

SOP	Standard operative procedure
G6PD	Glucose-6-Phosphate Dehydrogenase
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
EDTA	Ethylenediaminetetraacetic acid
QC	Quality Control
RPM	Revolutions Per Minutes
U/g Hb	Units per gram of hemoglobin
D.W	Distilled water

1. **Purpose**

   This standard operating procedure (SOP) document will provide a guide on how to perform quantitative Glucose-6-Phosphate Dehydrogenase (G6PD) testing using Mindray 240 pro Machine to ensure consistent and accurate results.

2. **Scope**

   This document is applicable to qualified and trained technical staff involved in the G6PD testing process using the Mindray 240 pro Machine within the laboratory.

3. **Definitions**

   Quantitative test is a test that provides a specific numerical value for a measured quantity.

4. **Policy**

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

   4.2 Pediatric patients with low packed red cells, clotted and hemolyzed samples should be rejected.

   4.3 Samples should be performed within 36 hours after collection.

   4.4 No male patient should be reported as Partial activity, on these samples' retic should be done and ask for repeat sample after drop of retic count.

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

5. **Procedure**

   5.1. Introduction

   The Glucose-6-Phosphate Dehydrogenase (G6PD) test measures the activity of the G6PD enzyme, which is a critical enzyme in the pentose phosphate pathway. This enzyme helps maintain red blood cell integrity by protecting it from oxidative damage. It does so by catalyzing the first step in the pentose phosphate pathway, which produces NADPH (Nicotinamide Adenine Dinucleotide Phosphate), a key molecule in defending against oxidative stress.

   5.2. Principle

   The G6PD test is based on the enzyme-catalyzed reduction of NADP+ to NADPH by the enzyme G6PD in the presence of glucose-6-phosphate. The production of NADPH is proportional to G6PD activity and is measured through a colorimetric change detected at 340 nm. Reduced enzyme activity (as seen in G6PD deficiency)

results in less NADPH and a lower rate of absorbance change, indicating enzyme deficiency.

This method allows for the detection of G6PD deficiency, a condition where the enzyme activity is low, leading to hemolytic anemia under stress conditions (e.g., certain infections, drugs, or foods).

5.3. Health & safety

Universal precautions must be practice at all stages of test.

5.4. Sample requirement

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

5.4.2. For pediatric samples, suspend 20 ul of the centrifuged erythrocytes in 1.0ml of distilled/ deionized water and mix fully for 2-5 min at 3000rpm.

5.5. Items required

5.5.1. Pipette tips.

5.5.2. Calibrated micropipettes

5.5.3. Test tubes or cuvettes.

5.5.4. Mindray G6PD test reagent kit

5.5.5. G6PD Normal control

5.5.6. G6PD Deficient control

5.5.7. Gauze

5.5.8. Deionized water

5.5.9. Ethanol

5.5.10. Centrifuge

5.6. Equipment Maintenance

5.6.1. Daily maintenance (Recommended daily prior to analysis):

a) Checking probe/Mixer/wash well:

- Select Utility > Maintenance > Biochemistry Maintenance. Select “Clean Probe/Mixer/Wash well”.
- Check the exterior of the probe and mixer for stains. If stains exist, perform the "Cleaning probe exterior" and "Cleaning the mixer" procedures.

- Select Continue to clean the probe interior and check the liquid flow of the probe. Refer to Figure 1 in annexes.
- Observe the water flow of the probe/mixer wash wells and check if the water reaches to about 5 mm of the probe/ mixer from the tip.
- Select Second Wash. The probe interior wash can be performed again.
- Select Continue and Done

b) Checking D.W tank and tube connection:

- Check the Distilled water tank and ensure enough deionized water available.
- Check that the tubes are not bent, folded or leaking.

c) Checking diluted wash solution tank and tube connection:

- 9:1 dilution is made to prepare Wash solution (1 L of detergent is mixed in 9L of distilled water)
- Check that the tubes are not folded and not leaking.

d) Checking waste connection

- Check the high-/low-concentration waste tubes and ensure that they are not leaking or bent.
- Empty the high-/low-concentration waste tanks if they are full.

e) Checking probe wash solution.

- Check the volume of the probe wash solution on the sample/reagent carousel position 49#.
- If necessary, fill more or replace the wash solution.

f) Cleaning electrode tubes

- Select Utility > Maintenance > Maintenance > ISE Maintenance.

- Choose Clean Electrode Tubes. The maintenance guide window shows.
- Open the upper protective shield of the analyzer.
- Fill a reagent bottle with at least 2.5 mL ISE wash solution and then load it to ISE wash solution position (Position 48#) on the sample/reagent carousel.
- Select Continue. The system starts cleaning the ISE electrode tubes.
- Select Done.

g) Check the Syringe.

- Check the T-piece assembly and plunger guide cap for leaks.
- Use dry gauze to wipe the T piece and then check if the gauze is moistened.

5.6.2. Weekly maintenance

a) Clean Probe exterior:

- Power off the analyzer
- Pull the probe arm up to its highest point and rotate it to a convenient position for cleaning.
- Use a clean gauze moistened with ethanol to clean the probe's exterior and tip.
- Use a gauze moistened with deionized water to clean the ethanol on the probe.
- After finishing the cleaning, close the shielding cover, and turn on the analyzing unit power switch.
- Select Utility > Commands > Home to reset the system.

b) Clean probe mixer:

- Power off the analyzer
- Pull the Mixer arm up to its highest point and rotate it to a convenient position for cleaning.
- Use a clean gauze moistened with ethanol to clean the mixer exterior and tip.
- Use a gauze moistened with deionized water to clean the ethanol on the mixer.

c) Special Wash

- Place more than 40 ml of concentrated wash solution in position D (Position 49) of the reagent carousel.
- Check the volume of the Deionized water tank.
- Select Utility > Maintenance > Maintenance > Biochemistry Maintenance and select “Special Wash”.
- Select Continue. To initiate the special wash cycle. After the cleaning, the system performs a cuvette check automatically.

5.6.3. Monthly maintenance:

a) Photometer check.

- Select Utility > Maintenance > Maintenance > Biochemistry Maintenance.
- Choose Photometer Check.
- Select Continue and the select Start.
- When the test is finished, check the test results.
- Check the results of the previous and current photometer check to understand the lamp status.
- Select Done to close the Photometer Check window.

5.7. Reagent

a) Reagent notes:

- Do not mix different reagents from different lots.
- Do not use expired reagents.
- Avoid direct exposure to sunlight and freezing. The results cannot be assured when stored at inappropriate condition.
- Reagent contamination must be avoided; use clean pipette tips for every reagent and sample.
- Store all reagents at 2-8C in closed containers when not in use.
- Do not store opened reagents for any longer than 21 days
- Discard reagents that have become turbid or discolored.

b) Reagent preparation:

R1: Ready to use

R2: Ready to use

Reagent 1 (R1)	Tris buffer Sodium chloride Preservative	200 mmol/L 5 g/L 0.5 g/L
Reagent 2 (R2)	Tris buffer Glocose-6-phosphate NADP Preservative	200 mml/L 10 mmol/L 10 mmol/L 1 g/L
Wash D	Detergent CD80 Distilled water	1 L 9 L

c) Storage and stability

- Up to expiration date indicated on the label, when store unopened at 2-8 C and protected from light.
- Once opened, the R1 and R2 reagent are stable for 21 days when refrigerated on the analyzer or refrigerator.

- Contamination of the reagent must be avoided.
- Do not freeze the reagents.

d) Checking reagent carousel status

- On the Reagent > Reagent Carousel Status screen, you can check the detailed information of each reagent.
- To view detailed information about a reagent, select its position on the carousel graph. The information will be displayed on the light side of the screen.

5.8. Calibration/ Quality Control

5.8.1. G6PD reagent/ Calibration screen:

- Select Reagent > Reagent/ calibration.
- Select chemistries you want to calibrate
- Select Cal F5 and select OK. If you decided to abort the calibration requests, select No Cal F6.
- Calibration tests can be cancelled only when they have not been started or are interrupted.
- Select start. The start conditions window in displayed.
- Select OK to start analysis.

5.8.2. Quality Control:

- The commercial control provided by the manufacturer.
- Run quality control samples (both Normal and Deficient) using the Mindray Machine prior to patient samples.
- Once opened, the R1 and R2 reagent are stable for 21 days when refrigerated on the analyzer or refrigerator.
- Document the results to ensure the machine is functioning correctly before running patient samples.

5.8.3. Procedure Steps

5.8.4. Allow all the reagent to reach room temperature before testing.

5.8.5. Centrifuge the sample under rotation speed 2000 RPM for 5 minutes (for on-board hemolysis).

5.8.6. Centrifuge the sample under rotation speed 3000 RPM for 5 minutes (for manual hemolysis).

5.8.7. Ensure patient samples are correctly labeled with identification numbers.

5.8.8. Load the prepared reagents onto the analyzer's reagent tray. Mindray's own G6PD reagents are available and are specifically designed for their BS series analyzers.

5.8.9. Place the prepared samples onto the sample tray. The BS-240 Pro has a flexible sample loading capacity.

5.8.10. The analyzer automatically aspirates the sample and reagents into a cuvette. The minimum reaction volume is 100 µL.

5.8.11. The analyzer incubates the reaction at **37°C** and measures the change in absorbance over a fixed time. The rate of change in absorbance is used to calculate the G6PD activity.

5.8.12. The results are then reported in U/g Hb (Units per gram of hemoglobin), which requires a separate measurement of the sample's hemoglobin concentration.

5.8.13. Once the test run is complete, print and record the results.

5.8.14. Figure 2 and 3 in annex show how the sample is processed.

5.9. Results

5.9.1. Interpret the results according to the reference ranges provided by Royal hospital

5.9.2. Document any deviations or issues encountered during the test run.

6. Responsibility

1. Laboratory Program committee:
    - Ensure that document is approved by ministry of Health and distributed to all institutions
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
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
4. Technical staff
    - Be familiar with this document and adhere to it where applicable

7. Document History and Version Control

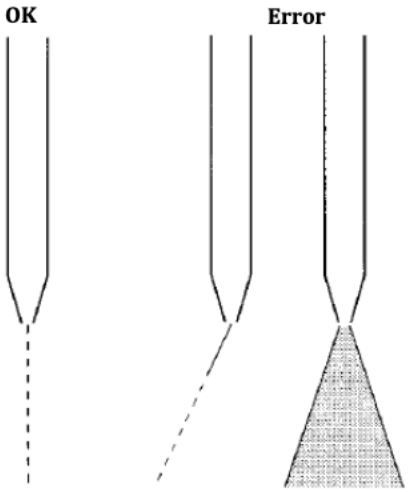
Version	Description	Review Date
1	Initial Release	August 2031

8. References

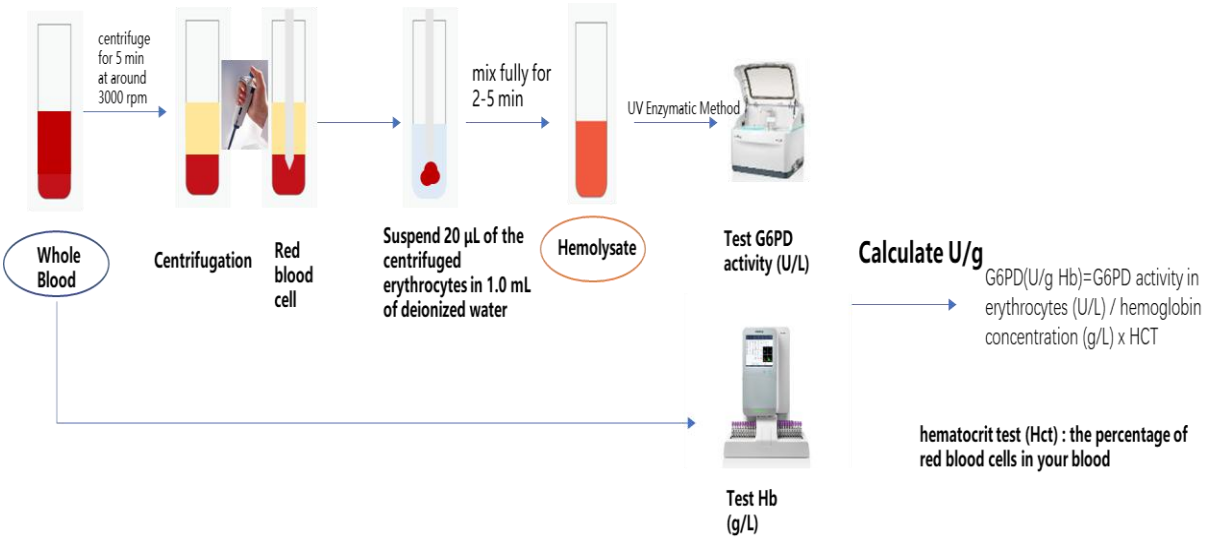
- Manufacturer's manual for the Mandry Machine.
- G6PD test kit instruction.

9. Annexes

- Figure1: Normal and abnormal liquid flows of the probe



- Figure2: Non-twin Test G6PD test (Manual hemolysis):



- Figure3: Twin test G6PD testing (On-board Hemolysis):