PATH	Standard Operating Procedure	
Document No:	**Version Number:** **00**	**Date signed:** **[Effective Date]**
Title:	**Flow Cytometry Assay for G6PD Analysis in Red Blood Cells**	

1. Purpose

Whole blood specimens will be characterized for intracellular G6PD activity by flow cytometry method as described here. This method allows observation of mosaic red blood cell populations in specimens from females by looking at the activity of G6PD in individual erythrocytes.

2. Scope

This SOP allows observation of mosaic red blood cell populations in specimens from females by looking at the activity of G6PD in individual erythrocytes. This methodology helps establishing a specimen panel for evaluation of G6PD POC tests.

3. Responsibilities

3.1. The Project lead has the authority to establish this procedure.
3.2. The Scientific lead is responsible for the control of SOP documentation.
3.3. Laboratory staff is responsible for the implementation of this procedure and for ensuring that all appropriate personnel are trained.

4. Health and Safety Information

4.1. Consider all patient specimens as capable of transmitting infectious agents. Use appropriate precautions such as gloves, safety glasses, and laboratory coats when collecting, handling, and disposing of patient specimens.
4.2. Dispose of all specimens and used materials in accordance with local applicable guidelines and/or regulations.
4.3. Refer to MSDS for the Trinity G6PDH assay more information.

5. Required Equipment

5.1. Eppendorf centrifuge
5.2. Heat block

5.3. Vortexer
5.4. Fume hood
5.5. Flow Cytometer
5.6. Pipettors: p10, p200, p1000 with tips

6. Required Material

6.1. Sodium Nitrite (0.125M) Sigma: S2252-500g, Dissolve in water
6.2. Phosphate Buffered Saline(PBS), Sigma: P4417-100TAB
6.3. Glucose (0.25M), Aldrich:158968-500g; Dissolve in PBS
6.4. Nile Blue Sulfate (0.01%), Sigma: N5632-25g; Dissolve in water
6.5. Potassium Cyanide (0.4M), Sigma: 60178-25g; Dissolve in water store @4C in dark. NOTE: Highly poisonous, handle with extreme care in fume hood.
6.6. 3% Hydrogen Peroxide, Fluka: 16911-250ml; Dissolve in PBS
6.7. Micro-centrifuge Tubes, 1.7ml, VWR: 211-0007
6.8. Additive: 2.5% Glucose, 0.9% Sodium Chloride, 0.27% Adenine, 0.75Mannitol

7. Procedure

7.1. Place 100 ul of undiluted and diluted 1:10 samples of EDTA + Additive blood in eppendorf tubes.
7.2. Mix with 100 ul of 0.125M sodium nitrite and incubate at room temperature for 20 minutes.
7.3. Wash samples three times with PBS, 2500rpm, 3 minutes.
7.4. Resuspend in 100 ul of PBS.
7.5. Add 18 ul of 0.28M glucose (PBS) and 6 ul of 0.01% Nile blue sulfate to each tube.
7.6. Incubate samples at 37C for 90 minutes in an aerobic environment, with the lids open.
7.7. Add 2.5 microliters of 0.4M potassium cyanide to each sample and incubate at room temperature for 5 minutes(fume hood).
7.8. Add 5 ul of each sample to 100 ul of 1:10 dilution of hydrogen peroxide and agitated vigorously by hand (fume hood).
7.9. Wash samples two times with PBS (fume hood).
7.10. Resuspend cells in 500 ul of PBS.
7.11. Analyze samples in a Flow cytometer using the FITC channel. Use normal red blood cells as control.

8. Related SOPs

8.1. Sample receiving, handling and data management

8.2. Use and Maintenance of Liquid Nitrogen storage tank

8.3. Sample shipment from specimen repository

SIGNATURES:

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Date

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