PATH	Standard Operating Procedure	
Document No: SPR-xx	**Version Number:** **00**	**Date signed:** **[Effective Date]**
Title:	**Trinity Glucose-6 Phosphate Dehydrogenase Fluorescent Spot Test**	

PURPOSE:

The Trinity Glucose-6 Phosphate Dehydrogenase Fluorescent Spot Screening test is used for the qualitative, visual fluorescence screening of G-6-PD activity in human blood samples.

SCOPE:

This SOP applies to Fluorescent Spot Screening of human red blood cells specimen for determining G6PD enzyme activity for successful development of G6PD Point of care diagnostic tests.

RESPONSIBILITIES:

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

PROCEDURES:

1. Specimen receiving

1.1. Blood samples from Bioreclamation and other collaboration sites will be received.

1.2. Whole blood with anti-coagulant will be received in cold chain at 2-8C.

1.3. Laboratory data management system *viz. Freezer Works* will be used for chain of custody.

2. Specimen Handling

2.1. Consider all human specimens as capable of transmitting infectious agents.

2.2. Use Blood borne pathogen precautions for all samples.

3. Specimen Rejection

3.1. Quality of specimens must be evaluated at the point of delivery.

3.2. Technicians must have appropriate laboratory training to assess quality before the entry of specimens into the processing workflow

3.3. **Unacceptable specimen criteria**

3.3.1. Unlabeled or mislabeled specimens must be rejected.
3.3.2. Clotted specimen must be rejected.

4. Personal Protective Equipment (PPE)

Personal protective equipment must be used for handling specimens and reagents.

4.1. Laboratory coat or gown

4.2. Eye protection

4.3. Latex or nitrile gloves, non–powdered preferred

5. Equipment, material and reagents

5.1. Trinity G6PD Fluorescent Spot test, P/N 203-A

5.2. Whatman No. 1 Filter Paper, Cat. No. 1001-150

5.3. Pipettes and tips capable of accurately dispensing 10 µl, 200 µl, and 1mL.

5.4. 1.5mL Eppendorf snap close tubes

5.5. Incubator set to 37°C

5.6. Digital timer

5.7. Spectroline Fluorescence Analysis Cabinet Model CM-10 (or similar equipment)

5.8. G6PDH deficient control, Trinity Biotech part # G5888

5.9. G6PDH intermediate control, Trinity Biotech part # G5029

5.10. G6PDH normal control, Trinity Biotech part # G6888

6. Preliminary Operations

6.1. Carefully read the product insert completely before using the test. Use the test only in accordance with the instructions contained in the product insert. Failure to do so may result in inaccurate test results.

6.2. Do not use the kit contents after the expiration date listed on the kit.

6.3. Allow water bath to pre-warm for at least 30 minutes.

6.4. Prepare G6PD Substrate Solution by adding a volume of TRIZMA Buffer Solution indicated on the label (2mL) to the reagent vial. Swirl gently and invert several times.

6.5. The assay can be calibrated when needed by testing deficient, intermediate, and normal controls, provided by Trinity Biotech. To test the controls, first rehydrate with 0.5 ml pure water. Mix well, and visually verify blood has been rehydrated before using. Follow the procedure with controls exactly as with whole blood specimens. Compare G6PD activity by visually comparing fluorescence levels with the expected visual fluorescence levels provided by Trinity Biotech.

7. Procedure

7.1. Add 10µL of whole blood (with anticoagulant) to tube containing 200µl of G6PD Substrate solution and mix. Promptly transfer drop of solution to filter paper. Identify spot with sample number and Time-Zero. Note: spots should be approximately ½ inch in diameter.

7.2. Place tube in 37°C water bath for 5 minutes.

7.3. After 5 minutes have elapsed remove tube from water bath and transfer a drop of solution on to filter paper next to original spot. Identify spot with sample number and Time – 5 minutes.

7.4. Place tube in 37°C water bath for 5 minutes.

7.5. After 5 minutes have elapsed remove tube from water bath and transfer a drop of solution on to filter paper next to other two spots. Identify spot with sample number and Time – 10 minutes.

7.6. Repeat steps above for all other samples and Normal, Intermediate, and Deficient controls.

7.7. Allow filter paper to dry for 20 minutes before visually inspecting spots under long-wave ultraviolet light. Record fluorescent intensity (absent, weak, moderate, or strong) of each sample at each time point and classify as deficient, intermediate, or normal enzyme activity according to level of fluorescence.

Notes:

- Because of the rapid speed of reaction, Zero-Time spots may exhibit traces of fluorescence.
- Fluorescent spots are stable for up to two weeks stored in a plastic bag with desiccant in the refrigerator at 2-8°C
- Visual comparison examples of controls are included in the kit insert.

8. Storage

8.1. All reagents should be refrigerated (2-8° C) when not in use. Reconstituted assay solution is stable for 5 days refrigerated

9. Related SOPs

9.1. Sample receiving, handling and data management

SIGNATURES:

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Date

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Date

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Date