CURRENT

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Title: Glucose 6 Phosphate Dehydrogenase (G6PD) analysis.
SOP No.: 13/01
Date first effective: 1st January 2025 **Next Review date:** 31st December 2025
Department of Clinical Pharmacology, 1st Floor, New MS Building,
Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012

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1. **Purpose**

The purpose of this Standard Operating Procedure (SOP) is to outline the procedure for in vitro determination of activity of Glucose 6 Phosphate Dehydrogenase (G6PD) from the red blood cell hemolysate in Biochemistry laboratory of Department of Clinical Pharmacology.

2. **Scope**

This SOP covers the procedure of analysis of the activity of Glucose 6 Phosphate Dehydrogenase (G6PD) in red blood cell hemolysate.

3. **Responsibility**

Lab technician, Lab attendant, or any other appropriately qualified staff in the team, delegated by the Head of Department, will be responsible for analysis.

4. **Reference to other applicable SOPs**

- Departmental SOP No. 10/04 Blood Collection.
- Departmental SOP No. 24 /03 Waste management
- Kit insert.

5. **Detailed Instruction**

1. The whole blood sample is collected in labeled EDTA bulb as per Departmental SOP No. 10/04.
2. This kit used for G6PD analysis is kept in Refrigerator located in the Biochemistry laboratory of Department of Clinical Pharmacology, M.S. Building, and 1st Floor.

Use this kit: G-6PD TEST KIT

KIT Expiry: 2 years after the manufacturing date mentioned on kit

Manufactured by :- Span Diagnostics Limited

3. **Working Reagent Preparation :-**
   - i. G6PD **1: Substrate**
   - ii. G6PD **2: Buffer, pH 8.5**
   - iii. G6PD **3: Lysing reagent**
   - iv. G6PD 4 **: Inert Oil**

The working reagent **(R1)** is prepared by reconstituting the contents of one vial of G6PD 1(Substrate) with 0.5 ml of G6PD 2(Buffer) in the same vial of G6PD 1.

4. Working reagent stability
   - G6PD **1 and 2** are stable for 30days at 2-8°C, when protected from contamination.
   - G6PD **3** can be stored at Room Temperature.
   - It is recommended to prepare fresh working reagent before assay is performed.
5. Prepare red cell hemolysate by adding 0.05ml of EDTA whole blood sample to 1 ml pre-cooled lysing reagent.
6. Transfer completely the whole blood hemolysate to the freshly prepared working reagent and shake well.
7. Immediately overlay 1 ml of G6PD 4 (inert oil)
8. Seal the vial tightly using plug and cap to make it air tight,incubate at 37°C.
9. Observe the change of initial blue to brownish colour.
   - **G6PD normal subjects:** Decolorization time is 30-60 minutes.
   - **G-6PD deficient subjects:** (Heterozygous males, homozygous females): 140 minutes to 24 hours.
   - **G-6PD carriers (Heterozygous females):** Some give results which overlap with normal males; others decolorize between 90 minutes and several hours.

10. Enter the value in form sent with the sample by concerned doctor. The form is attached in Appendix 1 as in biochemistry lab SOP no. 07/01.
11. Values are evaluated and signed by biochemist / laboratory in charge, study coordinator and PI.
12. Caution:
    - Blood with high reticulocyte count may give false normal results if it is enzyme deficient because reticulocytes generally have higher G-6PD activity than adult red cells.
    - Some samples may reach the end point and then slowly turn blue again due to re-oxidation of the dye. Hence it is necessary to observe reaction mixture at every 30 minutes interval.