G-Six
TEST FOR SCREENING AND QUANTITATION OF G6PD DEFICIENCY
(For in vitro diagnostic use only)

SUMMARY
Glucose-6-Phosphate-Dehydrogenase (G6PD) deficiency is one of the most common human enzyme deficiency in the world. During G6PD deficiency, the red cells are unable to regenerate reduced nicotine adenine dinucleotide phosphate (NADPH), a reaction that is normally catalyzed by the G6PD enzyme.
Since the X chromosome carries the gene for G6PD enzyme, this deficiency mostly affects the males.
The two major conditions associated with G6PD deficiency are hemolytic anaemias and neonatal jaundice, which may result in neurological complications and death. Screening and detection of G6PD deficiency helps in reducing such episodes, through appropriate selection of treatment, patient counselling and abstinence from disease precipitating drugs such as anti malarials and other agents.

REAGENTS
G-SIX test is a ready to use, three component reagent system of the detection of G6PD deficiency in human blood using the WHO recommended methemoglobin reduction method. The test system contains three vials (P), (T) and (N) predispensed with appropriate reagents along with Quantitation graph paper.
Each batch of the reagent undergoes rigorous quality control at various stages of manufacture for its sensitivity and performance.

STORAGE & STABILITY
1. Ideally the product should be stored at 2-8°C. It may also be stored between 20-25°C in a cool dark place away from light and moisture.
2. The shelf life of the reagent system is as per the expiry date mentioned on the G-SIX carton.

PRINCIPLE
The G-SIX test is based on the principle of reduction of methemoglobin by G6PD activity of the red cells under test. The rate of reduction is proportional to the G6PD activity of the red cells under test. During the test procedure the test sample is processed in triplicate so as to simultaneously also derive positive and normal reference controls. During screening method the color of the test sample is compared visually to the reference controls in order to arrive at the diagnostic conclusion. Quantitation of the percentage of G6PD deficiency can also be done spectrophotometrically.

SAMPLE COLLECTION & PREPARATION
Fresh whole blood sample collected in EDTA or Heparin only. The samples must be used within one hour of collection, since the G6PD enzyme actively decreases on storage at 2-8°C.
Blood samples may be collected in ACD and can be stored up to 7 days at 2-8°C before performing the test.
No special preparation of the patient is required prior to sample collection by approved techniques.
If the haematocrit of the sample is less than 30%, enough plasma should be removed from the sample to bring back the PCV to 40% ± 5%.

SCREENING TEST PROCEDURE
1. Open the pack of the reagent vials P(for test reference), T (for test reference) and N (for normal reference). Mark patients ID on the three vials. Use immediately upon opening.
2. Add 1 ml of the blood sample under test to each of the vials P, T and N, and mix well by gentle inversion.
3. Recap the vials tightly using the screw cap (the plug may be discarded) and place them vertically in an incubator which has already been stabilized at 37°C.
4. Incubate undisturbed, at 37°C, for 3 hours.
5. Meanwhile set up three 5 ml test tubes on a test tube stand and dispense 5 ml distilled / deionised water into each of these tubes.
6. Label these reference tubes as PR, TR and NR respectively and mark patient ID on each tube. (if more number of samples are being run simultaneously set up equivalent number of such distilled / deionised water tube sets.)
7. Remove the vials after 3-hour incubation and mix gently.
8. Uncap the incubated test vials P, T and N and dispense exactly 0.05 ml of the well mixed incubated samples using different pipettes into the corresponding appropriately labelled distilled water reference tubes PR, TR and NR.
10. Observe and compare the colour of tube TR with PR and NR against light to interpret the results.
11. The test results must be interpreted within three hours of preparation of tubes PR, TR and NR for screening test and within 30 minutes for the quantitative procedure.

**QUANTITATIVE PROCEDURE**

- **Wavelength / filter**: 505 nm (Hg546nm)/blue green
- **Temperature**: R.T.
- **Light path**: 1 cm

1. Dispense / aspirate required amount of NR as obtained in point no. 9 of screening procedure into the cuvette, NR serves as blank.
2. Similarly read the O.D. of PR and place the corresponding value on the G-SIX quantitation graph paper, which equates to 100% deficiency on the Y-axis.
3. Make a straight line joining the blank value (0.00) and the O.D. of PR.
4. Read the O.D. of TR and place it on the graph paper.
5. Find out the % G6PD deficiency, corresponding to the O.D. value of TR on the Y axis of the graph paper.

**INTERPRETATION OF RESULTS**

**SCREENING TEST**

- Normal Sample: Tube TR has a clear red colour, matching with the normal reference tube NR.
- G6PD Deficient Sample: The test tube TR has a brown colour matching with the positive reference tube PR.

(Full expression) G6PD Deficient Sample: The tube TR has intermediate colour as compared (Intermediate females) to positive reference tube PR and negative reference tube NR depending on the degree of expression of the deficiency trait.

**QUANTITATIVE TEST**

<table>
<thead>
<tr>
<th>Class of Deficiency</th>
<th>% of G6PD Deficiency</th>
<th>Clinical Relevance</th>
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</thead>
<tbody>
<tr>
<td>CLASS I</td>
<td>Complete</td>
<td>Chronic, congenital nonspherocytic, anaemia without drugs/oxidative stress</td>
</tr>
<tr>
<td>CLASS II</td>
<td>90% or more</td>
<td>Acute hemolytic crisis induced by oxidative drugs</td>
</tr>
<tr>
<td>CLASS III</td>
<td>40%-90%</td>
<td>Oxidative drugs/ infection induces self-limiting hemolysis without previous hematologic disorder</td>
</tr>
<tr>
<td>CLASS IV</td>
<td>Less than 40%</td>
<td>Associated with milder clinical conditions, depending on the variant involved</td>
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</table>

% G6PD deficiency of upto 20% as obtained by G-SIX test, corresponds to the normal range of 4.5 - 13.5 U/g Hb activity.

**REMARKS**

1. Do not expose the reagents during storage or during test to direct sunlight.
2. Before performing the test if the reagent vials show any moisture or condensation on the inner walls; they must be discarded. use another set for conducting the test.
3. The reagent vials should be used immediately after opening.
4. Young red cells have a higher G6PD content than the older ones, regardless of the genetic variant that is present. If the enzymes have defective activity, older cells are preferentially destroyed during mild to moderate hemolytic phase. Since reticulocytes released to replace lost cells have high enzyme levels, false negative results may occur if blood is tested immediately after a hemolytic episode.
5. The blood should be tested within an hour of collection as recommended. Delay in testing may give rise to false positives.
6. It is extremely important that the 5 ml test tubes used for post incubation sample dilution are free from acids or alkalies as this may interfere with end colour stability.
7. Transfer of correct samples to the correctly labelled reference tubes PR, TR and NR is extremely vital for achieving correct results.
8. Vitamin C supplements or a large dietary intake of vitamin C may interfere with the reaction.
9. The positive reference PR must be a brown colour. The negative reference must have a cherry pink to cherry red
colour. These colours must be achieved to validate test run and correct transfer of incubated samples to correct and corresponding reference tubes.

10. If the positive and negative reference tubes (PR and TR) have a different colour than expected the test must be re-run. It must be noted however that the test reference will show varying colours from red to brown depending upon the degree of G6PD deficiency in the sample.

REFERENCES