QUANTITATIVE DETERMINATION OF G6PD IVD

ORDER INFORMATION

REF: G6PDS 10 Cont. 1X10 Tests

CLINICAL SIGNIFICANCE OF G6PD TEST

G6PD deficiency is the underlying cause of certain drug induced Hemolytic anaemia.

PRINCIPLE

GLUCOSE-6-Phosphate Dehydrogenase present in the red cell hemolysate, acts on Glucose-6-Phosphate and reduces NADP which, in the presence of PMS, reduces the blue colored 2,6 Dichlorophenolindophenol into colorless form leaving behind the original cherry red color of the hemolysate. The Rate of decolorisation is proportional to the enzyme activity.

REACTION

\[ \text{G6PDH} \]
\[ \text{G-6-Phosphate} + \text{NADP} \Leftrightarrow 6 \text{ - Phoshogluconic Acid} + \text{NADPH} \]

NADPH + 2, 6 Dichlorophenol Indophenol Blue \Leftrightarrow NADP + Reduced 2, 6 DCPIP (Colorless).

Rate of Decolorisation \( \propto \) G-6PD Activity.

SAMPLE

Whole blood collected in E.D.T.A bulb.

REAGENTS

1. SUBSTRATE VIALS
2. BUFFER , Ph 8.5
3. MINERAL OIL

REAGENT STORAGE & STABILITY

Reagent 1 & 2 are to be stored at 2-8°C. All reagents are stable still expiry date mentioned on the vials when stored at the proper storage conditions.

PRECAUTIONS

Use fresh blood samples, as enzyme activity reduces on refrigeration. Do not use Heparin as an anticoagulant, Heparin interferes with the results. Run one normal non deficient sample with each batch.

WORKING REAGENT PREPARATION

Bring all the reagents to room temperature. Tap the substrate vials gently on a flat surface to dislodge all the substrate powder. Just before use, using clean pipettes, reconstitute each substrate vial with 0.5 ml of Buffer using clean pipettes, reconstitute each substrate vial with 0.5 ml of Buffer Reagent. Gently swirl to dissolve and allow to stand for five minutes.

PREPARATION OF RED CELL HEMOLYSATE.

Enclosed below is table showing amount of blood required to prepare the hemolysate corresponding to the Hemoglobin concentration (G/DL).

<table>
<thead>
<tr>
<th>Hemoglobin Conc. GM/DL</th>
<th>Quantity of Blood (ml)</th>
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</thead>
<tbody>
<tr>
<td>7.0 to 9.50</td>
<td>0.04 ml</td>
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<tr>
<td>9.60 to 11.50</td>
<td>0.03 ml</td>
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<tr>
<td>11.6 to 13.50</td>
<td>0.025 ml</td>
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<tr>
<td>13.6 to 15.0</td>
<td>0.02 ml</td>
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</tbody>
</table>

in 1.0 ml of Distilled water add 20 μl (0.02 ml) of well mixed E.D.T.A. whole blood sample. Mix well & allow to stand for 5 minutes at room temperature.

ASSAY PROCEDURE

1. Add 1 ml of the hemolysate to the reconstituted substrate vial & mix gently by swirling.
2. Add immediately 1 ml of the mineral oil.
3. Replace the plug & the cap tightly. Incubate undisturbed at R.T. for 60 minutes in dark.

NOTE: The vials should be incubated undisturbed as the introduction of air from the atmosphere will allow the blue color to reappear & will give erroneous results.

INTERPRETATION OF RESULTS

DECOLORISATION TIME

Normal subjects: 5 to 60 minutes.
G6PD Deficient subjects: ( In Heterozygous males & Homozygous females) Decolorisation time is 2 to 24 hrs.
In Heterozygous females who are carriers the cell population is mixed with normal & deficient cells. The distribution of deficient cells varies from individual to individual ranging from 20% to 80%. Hence some such subjects may give results overlapping over normal as well as abnormal time specifications i.e. the decolorisation in some hærerozygotes will be between 5-60 minutes (normal) & for some heterozygotes the same will be 2 hrs or more.

NOTES

1. Sample having hemoglobin content below 15 GM% proportionately use more amount of blood for preparation of the hemolysate.
2. Blood with high reticulocyte count may give false normal results even though the patient is enzyme deficient as reticulocytes generally have a higher G6PD activity than adult red cells. This is of very great importance if the test is carried out immediately after a hemolytic episode in a primaquine sensitive subject.

REFERENCE