GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY (Procedure No. 400)

INTENDED USE
Trinity Biotech. Glucose-6-Phosphate Dehydrogenase (G-6-PDH) reagents are for the qualitative, visual, colorimetric determination of G-6-PDH deficiency in red cells.

BACKGROUND AND PRINCIPLE OF TEST
A semi-quantitative procedure for measuring G-6-PDH (EC 1.1.1.49) in red cells was developed by Motulsky and Campbell-Kraut, using brilliant cresyl blue as an indicator. Subsequently, other workers replaced the dye with dichlorophenol indophenol. This method serves as the basis for the Trinity Biotech procedure for estimating red cell G-6-PDH.

G-6-PDH released from lysed erythrocytes catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconate with reduction of nicotinamide adenine dinucleotide phosphate (NADP) to NADPH. In the presence of phenazine methosulfate (PMS), NADPH reduces the blue dye, dichlorophenol indophenol, to the colourless form. The rate at which colour visibly disappears in the reaction mixture is proportional to the G-6-PDH content of red cells.

PROCEDURE:
1. Prepare red cell haemolysate, add 0.05 ml blood to 2.5 ml water. Shake gently and allow to stand for about 5 minutes.
2. Reconstitute Glucose-6-Phosphate Substrate vials with volume of TRIZMA Buffer Solution, Catalogue No. 400-4-50, indicated on the reagent box.
   a. For Five tests per assay vial (G-6-PDH Deficiency Substrate 5 x 10), 2.5 ml size.
   b. For Ten tests per assay vial (G-6-PDH Deficiency Substrate 10 x 10), 5.0 ml size.
3. When reconstituted according to directions, contains approximately the following concentrations of active ingredients:
   - Glucose-6-Phosphate: 4.6 mmol/L
   - NADP: 0.13 mmol/L
   - Dichlorophenol indophenol: 0.55 mmol/L
   - Also contains PMS.
4. Add 1.0 ml red cell hemolysate and shake gently to mix.
5. Also contains PMS.
6. Place vial or tube in 37°C water bath in subdued light. Note time.
7. The TEST should be observed at 15-minute intervals up to 1 hour for a change in colour from its original deep blue to a maroon or reddish endpoint. DO NOT AGITATE the reaction mixture during the incubation. Introduction of air will cause the blue colour to reappear. For normal blood, the initial deep blue colour of the reaction mixture will gradually reach the endpoint within 20-60 minutes. The endpoint can be more readily detected if the vial or tube is viewed horizontally in front of a bright light. There may be a thin blue layer remaining at the oil/aqueous interface due to trapped air which has oxidized reduced dye. This layer should be ignored when evaluating the endpoint. Viewing in front of a bright light will prevent the endpoint from being obscured by reflection from this blue coloured layer.

QUALITY CONTROL:
The reliability of test results should be monitored by the use of at least two levels of G-6-PDH Controls for this purpose: Deficient (Catalogue No. G5029) and Normal (Catalogue No. G6888). Completion times determined for these materials by this procedure should fall within the stated times of the controls.

LIMITATIONS:
This test is designed to distinguish normal from grossly deficient samples and should not be used to assess the degree of deficiency. It is recommended that samples requiring longer than 60 minutes to reach the endpoint (indicating G-6-PDH deficiency) be assayed by a quantitative G-6-PDH technique such as Trinity Biotech Procedure No. 345-UV.
EXPECTED VALUES
Blood collected from 102 clinically healthy adult males and females was assayed according to the described method. As shown in the table, all but one of the samples reached the endpoint within 1 hour.

<table>
<thead>
<tr>
<th>Endpoint (minutes)</th>
<th>0-20</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51-60</th>
<th>61-70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Samples</td>
<td>2</td>
<td>19</td>
<td>43</td>
<td>29</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

It should be noted that the expected values are based on incubation at 37°C. When other incubation temperatures are employed, the endpoint time may differ. For example, a normal specimen that reaches the endpoint in 30 minutes at 37°C may take longer at 25°C.

PERFORMANCE CHARACTERISTICS
A series of 5 replicate determinations were performed on blood samples collected from 3 adults. Dye decolorization times (minutes) for the 3 samples ranged from 28-31, 55-59 and 48-52.

CORRELATION:
The described visual colorimetric method is recommended because of its dependability in detecting G-6-PDH deficient ("primaquine-sensitive") individuals. Comparisons made with the methemoglobin reduction and ascorbate-cyadiate tests, have established its reliability.

TRIZMA is a registered trademark of Sigma-Aldrich Co., St. Louis, MO, USA.

BIBLIOGRAPHY
10. Data obtained by Trinity Biotech.

ORDERING INFORMATION

KITS
<table>
<thead>
<tr>
<th>Catalogue No.</th>
<th>400K-100-5 x 20</th>
<th>400K-100X</th>
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<tbody>
<tr>
<td>Maximum Assays Each Contains</td>
<td>100</td>
<td>100</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Description(Catalogue No.)</th>
<th>400K-100-5 x 20</th>
<th>400K-100X</th>
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</thead>
<tbody>
<tr>
<td>Substrate (400-5 x 10)</td>
<td>2 x 10 vials</td>
<td>0 vials</td>
</tr>
<tr>
<td>Substrate (400-10 x 10)</td>
<td>0 vials</td>
<td>10 vials</td>
</tr>
<tr>
<td>TRIZMA® Buffer (400-4-50)</td>
<td>50 ml</td>
<td>50 ml</td>
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<tr>
<td>Mineral Oil (400-5-100)</td>
<td>2 x 100 ml</td>
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INDIVIDUAL REAGENTS

<table>
<thead>
<tr>
<th>Catalogue No.</th>
<th>Item</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>400-5 x 10 (5 assays/vial)</td>
<td>Deficiency Substrate</td>
<td>10 x 2.5 ml</td>
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<tr>
<td>400-10 x 10 (10 assays/vial)</td>
<td>Deficiency Substrate</td>
<td>10 x 5 ml</td>
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<tr>
<td>400-4-50</td>
<td>TRIZMA® Buffer</td>
<td>50 ml</td>
</tr>
<tr>
<td>400-5-100</td>
<td>Mineral Oil</td>
<td>100 ml</td>
</tr>
<tr>
<td>400-5-1000</td>
<td>Mineral Oil</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

GUIDE TO SYMBOLS

- **REF**: Catalog number
- **LOT**: Batch code
- **ADD**: Contains sufficient for <n> tests
- **IVD**: For in vitro Diagnostic Use
- **Temperature limitation**: In vitro
- **Protect from light**: Use by

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400K-29 Rev E
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