Latest state of art in screening of G6PD deficiency
• Glucose-6-phosphate dehydrogenase (G6PD) is an important enzyme
  – plays a crucial role in protecting red blood cells (RBC) from oxidative damage
• Catalyses a reaction that produces NADPH
  – maintenance of reduced glutathione (GSH) pool used for detoxification of oxidative products (peroxides & superoxides)
  – GSH helps to keep sulfhydryl groups of Hb prot in the reduced state
In G6PD deficiency, oxidant stress → oxidative products accumulates & leads to:

- lipid peroxidation of RBC membrane
- denaturation of haemoglobin
  - Damage of RBCs membrane and RBC destruction (haemolytic anaemia)
G6PD DEFICIENCY
INHERITANCE

- Inherited in an X-linked recessive pattern.
- Males only have one X chromosome
  - one altered copy of the gene: severe disease.
- Females (two X chromosomes): Both copies of the gene involved - cause severe disorder.
Hemizygous male (X*Y) and homozygous female (X*X*) – full phenotypic expression

Heterozygous female X*X): expression variable due to Lyons phenomenon (normal, moderately deficient or severely deficient)
In female heterozygotes, red cell mosaicism arising from random X chromosome inactivation results in G6PD-deficient and G6PD-normal cell types.

The proportion of these two cells can vary enormously ranging from completely normal G6PD activity to complete deficiency.

Most female heterozygotes may have overall G6PD activity that can range from 10% to 60%, classified as moderate deficiency – will be missed by fluorescent spot test (FST).

Jiang WY, et al. (2015)
For many years, G6PD deficient heterozygotes were not regarded as being at risk.

However, many studies have shown that female heterozygotes are at risk of developing severe neonatal hyperbilirubinemia (Davidson et al, 1963; Meloni et al, 1983; Reclos et al, 2000).

And recently our own experienced handling jaundice female heterozygotes neonates, her molecular analysis showed heterozygosity for G6PD Mahidol 487G>A.

Therefore, heterozygotes should be warned as early in life as possible and treated as if she is totally G6PD deficient.
DIAGNOSIS OF G6PD DEFICIENCY

- FBC with reticulocytes count, peripheral blood smear:
  - Hb low with high reticulocytosis and PBF showed features of haemolytic crisis

- Qualitative assessment of G6PD enzymatic activity (UV-based test) – fluorescence spot test:
  - Screening for G6PD deficiency (in Malaysia since 1986)

- Quantitative measurement of G6PD enzymatic activity
  - Confirmatory test – exact measurement of G6PD enzymes activity

- Detection of G6PD gene mutations
  - Molecular test – real time PCR
### Table 3. Hematological data of the glucose-6-phosphate dehydrogenase (G6PD) deficient newborns jaundiced

<table>
<thead>
<tr>
<th>Number register</th>
<th>Hematological data</th>
<th>Reticulocyte count (%)</th>
<th>Direct Coombs test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Red blood cells (x10^{12} /L)</td>
<td>Hematocrit (%)</td>
<td>Hemoglobin (g/dL)</td>
</tr>
<tr>
<td>15</td>
<td>4.64</td>
<td>49.1</td>
<td>17.1</td>
</tr>
<tr>
<td>61</td>
<td>4.82</td>
<td>48.5</td>
<td>16.4</td>
</tr>
<tr>
<td>146</td>
<td>4.40</td>
<td>39.0</td>
<td>13.3</td>
</tr>
<tr>
<td>180</td>
<td>4.12</td>
<td>40.2</td>
<td>13.8</td>
</tr>
<tr>
<td>Mean</td>
<td>4.50</td>
<td>44.2</td>
<td>15.2</td>
</tr>
<tr>
<td>SD</td>
<td>0.30</td>
<td>5.3</td>
<td>1.9</td>
</tr>
</tbody>
</table>

SD = standard deviation
Reference values (3rd day after birth)^25: Red blood cell count: 5.3 ± 1.3 x 10^{12}/L; hematocrit: 56 ± 11%; hemoglobin: 18.5 ± 4.0 g/dL; reticulocytes: 1-4.5%.

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**Haemoglobin with reticulocytes count**

Marli Auxiliadora, et al, 2010
Peripheral blood smear of G6PD deficient patient in haemolytic crisis
Florescence spot test

- NADPH, generated by G6PD present in a lysate of blood cells, fluoresces under long wave UV light.
- G6PD deficiency - in sufficient production of NADPH → lack of fluorescence.
- Those with moderate G6PD activity will produce minimal NADPH
- Rapid, simple, inexpensive
- **Only detect when enzyme levels > 20%.**
- Not useful during haemolytic crisis – reticulocytes lead to false positive results in G6PD def individuals

Qualitative assessment of G6PD enzymatic activity

Florescence spot test

Results of G6PD screening test by FST method observe on chromatograph paper inside the UV box.
Quantitative measurement of G6PD enzymatic activity

- UKM Med Centre experienced, evaluating 4 types of G6PD assay kit:
  - G6PD kit assay from Randox Laboratories Ltd (Ainoon et al, 2003)
  - OSMMR-D G6PD kit assay from R & D Diagnostics (Holargos, Greece) (Azma et al, 2010)
  - G6PD kit assay from Mindray in 2013 (Norunaluwar et al, 2017)
- **Method**
  UV Enzymatic Method
Quantitative measurement of G6PD enzymatic activity

- Reaction Principle

NADP is reduced by G6PD in the presence of G-6-P. The enzyme activity is determined by measurement of the rate of absorbance change at 340 nm due to reduction of NADP.
The Randox kit utilizes the chemical reaction described by Beutler (1968), and the NADPH produced was measured at 340 nm in a kinetic mode using a Hitachi 717 (Boehreinger Manheim, Germany) autoanalyser.

The Hb concentration was measured on a Coulter Stac S cell counter.

G6PD activity was expressed in U/gHb using the following calculation and was calculated manually:

\[
\text{G6PDH U/gHb} = \frac{\text{G6PDH mU/erythocytes per ml}}{\text{Hb g/dl}} \times 1,000
\]
Table 1
G6PD activity levels for normal Malay and Chinese neonates.

<table>
<thead>
<tr>
<th></th>
<th>Mean G6PD activity U/gHb</th>
<th>Upper limit of total deficiency (20% of mean value U/gHb)</th>
<th>Upper limit of partial deficiency (60% of mean value) U/gHb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malay (n=669)</td>
<td>14.8</td>
<td>2.96</td>
<td>8.8</td>
</tr>
<tr>
<td>Chinese (n=307)</td>
<td>14.3</td>
<td>2.86</td>
<td>8.5</td>
</tr>
<tr>
<td>Total (976)</td>
<td>14.55</td>
<td>2.92</td>
<td>8.65</td>
</tr>
</tbody>
</table>

There was no significant difference in mean G6PD activity between male and female neonates in each racial group (p <0.05).

Table 4
G6PD activity in G6PD deficient neonates and overall frequency of G6PD-deficiency according to ethnic group, by semiquantitative and quantitative methods.

<table>
<thead>
<tr>
<th>G6PD deficiency</th>
<th>Ethnic Group (no. of neonates)</th>
<th>Range of G6PD activity U/gHb</th>
<th>Mean of G6PD activity U/gHb</th>
<th>No. (Frequency) diagnosed by semiquantitative method</th>
<th>No. (Frequency) diagnosed by quantitative method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Deficiency</td>
<td>Malay (n=669)</td>
<td>0-1.48</td>
<td>0.65</td>
<td>21 (3.1%)</td>
<td>21 (3.1%)</td>
</tr>
<tr>
<td></td>
<td>Chinese (n=307)</td>
<td>0-1.7</td>
<td>1.05</td>
<td>11 (3.6%)</td>
<td>11 (3.6%)</td>
</tr>
<tr>
<td>Partial Deficiency</td>
<td>Malay (n=669)</td>
<td>3.2-8.6</td>
<td>7.01</td>
<td>0</td>
<td>26 (3.3%)</td>
</tr>
<tr>
<td></td>
<td>Chinese (n=307)</td>
<td>2.89-8.17</td>
<td>6.0</td>
<td>0</td>
<td>12 (3.8%)</td>
</tr>
<tr>
<td>Total no of G6PD-deficient cases</td>
<td></td>
<td></td>
<td></td>
<td>32 (3.28%)</td>
<td>70 (7.7%)</td>
</tr>
</tbody>
</table>

Total number of neonates studied: 976

Ainoon et al, 2003
The **OSMMRD G6PD kit** assay (*Reclos et al, 1999*) is a method for measurement of G6PD activity that employs the haemoglobin normalization procedure.

This enzyme assay was introduced as routine service for neonatal screening in UKMMC in 2011, the only centre in Malaysia (screening for female neonates).

We evaluated this method before introduced for service and established the normal range for G6PD activity and cut-off points for G6PD deficiency for:

- normal term neonates and adults (*Azma et al, 2010*)
- paediatric age 1 month – 12 years (*Azma et al, 2014*)
The principle in measurement using OSMMR-D G6PD assay kit is the same as the Randox method.

Red cell G6PD activity is measured by the production of NADPH in the reaction mix. Measurement is done at 550nm using a spectrophotometer, and is directly proportional to the level of G6PD activity in the blood sample.

This is followed by measurement of Hb concentration again at 405 nm, hence allowing the simultaneous measurement of G6PD activity and Hb concentration.
G6PD enzymatic activity – Azma et al 2010

- Enzyme activity expressed in U/gHb Calculated using the following formula:

\[
\frac{(\delta \text{OD}_{\text{sample}550\text{nm}}/\text{min})}{(\delta \text{OD}_{\text{control}550\text{nm}}/\text{min})} \times \text{Control} = \text{sample} \ (\text{U/gHb})
\]

- The above calculation was done automatically by software that come together with the spectrophotometer (microplate reader EL808).
### Table 1
**G6PD activity levels for normal Malay and Chinese neonates.**

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>No. of cases</th>
<th>Mean G6PD activity (U/gHb)</th>
<th>Upper limit of total deficiency (20% of mean value) U/gHb</th>
<th>Upper limit of partial deficiency (60% of mean value) U/gHb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malay</td>
<td>48</td>
<td>12.44</td>
<td>2.49</td>
<td>7.46</td>
</tr>
<tr>
<td>Chinese</td>
<td>46</td>
<td>12.42</td>
<td>2.48</td>
<td>7.45</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>12.43</td>
<td>2.49</td>
<td>7.46</td>
</tr>
</tbody>
</table>

There were no significant differences in mean G6PD activity between both racial groups ($p > 0.05$).

### Table 2
**G6PD activity levels for normal male and female neonates.**

<table>
<thead>
<tr>
<th>Races</th>
<th>Gender</th>
<th>No. of cases</th>
<th>Mean G6PD activity (U/gHb)</th>
<th>Upper limit of total deficiency (20% of mean value) U/gHb</th>
<th>Upper limit of partial deficiency (60% of mean value) U/gHb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malay</td>
<td>Male</td>
<td>25</td>
<td>12.72</td>
<td>2.54</td>
<td>7.60</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>23</td>
<td>12.13</td>
<td>2.42</td>
<td>7.28</td>
</tr>
<tr>
<td>Chinese</td>
<td>Male</td>
<td>24</td>
<td>12.95</td>
<td>2.59</td>
<td>7.77</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>22</td>
<td>11.85</td>
<td>2.37</td>
<td>7.11</td>
</tr>
<tr>
<td>Total</td>
<td>Male</td>
<td>49</td>
<td>12.83</td>
<td>2.57</td>
<td>7.70</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>45</td>
<td>11.99</td>
<td>2.40</td>
<td>7.19</td>
</tr>
</tbody>
</table>

There were no significant differences in mean G6PD activity between males and females in each racial group ($p > 0.05$).
### Table 3

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>No. of cases</th>
<th>Mean G6PD activity (U/gHb)</th>
<th>Upper limit of total deficiency (20% of mean value) U/gHb</th>
<th>Upper limit of partial deficiency (60% of mean value) U/gHb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malay</td>
<td>151</td>
<td>9.43</td>
<td>1.87</td>
<td>5.66</td>
</tr>
<tr>
<td>Chinese</td>
<td>144</td>
<td>8.94</td>
<td>1.79</td>
<td>5.36</td>
</tr>
<tr>
<td>Total</td>
<td>295</td>
<td>9.20</td>
<td>1.84</td>
<td>5.52</td>
</tr>
</tbody>
</table>

There were no significant differences in mean G6PD activity between both racial groups ($p > 0.05$).

### Table 4

<table>
<thead>
<tr>
<th>Races</th>
<th>Gender</th>
<th>No. of cases</th>
<th>Mean G6PD activity (U/gHb)</th>
<th>Upper limit of total deficiency (20% of mean value) U/gHb</th>
<th>Upper limit of partial deficiency (60% of mean value) U/gHb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malay</td>
<td>Male</td>
<td>84</td>
<td>9.26</td>
<td>1.85</td>
<td>5.60</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>67</td>
<td>9.65</td>
<td>1.93</td>
<td>5.79</td>
</tr>
<tr>
<td>Chinese</td>
<td>Male</td>
<td>68</td>
<td>9.07</td>
<td>1.81</td>
<td>5.44</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>76</td>
<td>8.82</td>
<td>1.76</td>
<td>5.29</td>
</tr>
<tr>
<td>Total</td>
<td>Male</td>
<td>152</td>
<td>9.17</td>
<td>1.85</td>
<td>5.50</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>143</td>
<td>9.21</td>
<td>1.83</td>
<td>5.52</td>
</tr>
</tbody>
</table>

There were no significant differences in mean G6PD activity between males and females in each racial group ($p > 0.05$).
# G6PD Activity in Normal Adults

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of cases</th>
<th>Mean G6PD activity (U/gHb)</th>
<th>Upper limit of total deficiency (20% of mean value) U/gHb</th>
<th>Upper limit of partial deficiency (60% of mean value) U/gHb</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 - 29</td>
<td>74</td>
<td>9.60</td>
<td>1.92</td>
<td>5.76</td>
</tr>
<tr>
<td>30 - 39</td>
<td>78</td>
<td>8.84</td>
<td>1.77</td>
<td>5.30</td>
</tr>
<tr>
<td>40 - 49</td>
<td>68</td>
<td>9.02</td>
<td>1.80</td>
<td>5.41</td>
</tr>
<tr>
<td>50 - 59</td>
<td>75</td>
<td>9.34</td>
<td>1.87</td>
<td>5.60</td>
</tr>
<tr>
<td>Total</td>
<td>295</td>
<td>9.20</td>
<td>1.84</td>
<td>5.52</td>
</tr>
</tbody>
</table>

Table 5: G6PD activity in different age group in adults.

There were no significant differences in mean G6PD activity among different age groups in adults ($p > 0.05$).

Azma et al, 2010
Table 1: There were no significant differences in the mean normal G6PD activity among age groups.

*There were also no significant differences in the mean normal G6PD activity among races and among gender in each racial group.

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of cases</th>
<th>Mean G6PD activity (U/gHb)</th>
<th>Upper limit of total deficiency (20% of mean value)</th>
<th>Upper limit of partial deficiency (60% of mean value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month-1 year</td>
<td>24</td>
<td>9.64±3.34</td>
<td>1.93</td>
<td>5.78</td>
</tr>
<tr>
<td>1-2 year</td>
<td>37</td>
<td>10.85±2.94</td>
<td>2.17</td>
<td>6.51</td>
</tr>
<tr>
<td>3-4 year</td>
<td>34</td>
<td>10.55±3.18</td>
<td>2.11</td>
<td>6.33</td>
</tr>
<tr>
<td>5-6 year</td>
<td>24</td>
<td>9.73±3.76</td>
<td>1.95</td>
<td>5.84</td>
</tr>
<tr>
<td>7-8 year</td>
<td>33</td>
<td>10.05±2.42</td>
<td>2.01</td>
<td>6.03</td>
</tr>
<tr>
<td>9-10 year</td>
<td>26</td>
<td>9.85±3.60</td>
<td>1.97</td>
<td>5.91</td>
</tr>
<tr>
<td>11-12 year</td>
<td>36</td>
<td>10.17±3.84</td>
<td>2.03</td>
<td>6.10</td>
</tr>
<tr>
<td>Total</td>
<td>214</td>
<td>10.18±3.34</td>
<td>2.04</td>
<td>6.11</td>
</tr>
</tbody>
</table>

There were no significant differences in the mean G6PD activity among different age groups in children age 1 month to 12 years old (p>0.05).
### SUMMARY

<table>
<thead>
<tr>
<th></th>
<th>Neonates</th>
<th>Paediatrics</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Severe deficiency</strong></td>
<td>&lt; 2.5 U/gHb</td>
<td>&lt; 2.03 U/gHb</td>
<td>&lt; 1.84 U/gHb</td>
</tr>
<tr>
<td></td>
<td>(2.5 U/gHb is 20% of normal mean)</td>
<td>(2.03 U/gHb is 20% of normal mean)</td>
<td>(1.84 U/gHb is 20% of normal mean)</td>
</tr>
<tr>
<td><strong>Partial deficiency</strong></td>
<td>2.5 – 7.4 U/gHb</td>
<td>2.03 – 6.11 U/gHb</td>
<td>1.84 – 5.52 U/gHb</td>
</tr>
<tr>
<td></td>
<td>(7.4 U/gHb is 60% of normal mean)</td>
<td>(6.11 U/gHb is 60% of normal mean)</td>
<td>(5.52 U/gHb is 60% of normal mean)</td>
</tr>
<tr>
<td><strong>Normal mean G6PD</strong></td>
<td>12.43 ± 2.28 U/gHb.</td>
<td>10.18 ± 3.36 U/gHb.</td>
<td>9.21 ± 2.6 U/gHb.</td>
</tr>
<tr>
<td><strong>activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The above values have to be re-calculated every time our lab received new batch of reagent with new control value/lot number.

20% cut-off point was based on observation seen in previous studies by Ainoon et al showed that all severe def G6PD male neonates by FST, their enzyme activity < 20% of normal mean
The principle in measurement using this kit is similar as Randox kit.

The enzyme was measured at 340 nm in a kinetic mode using Mindray BS480 chemistry analyser.

The Hb concentration was measured on a Beckman Coulter DXH 800, automated blood cell counter.

G6PD activity was expressed in U/gHb using the following calculation and was calculated manually:

\[
\text{G6PDH U/gHb} = \frac{\text{G6PDH mU/erythocytes per ml} \times 1,000}{\text{Hb g/dl}}
\]
There were no significant differences in mean G6PD activity between Malay and Chinese groups \((p>0.05)\) for both tests but when compare mean G6PD activity between two tests, statistically significant \((p<0.05)\).
A correlation study between G6PD activities measured by Mindray Kit and OSMMR-D G6PD Assay Kit tests \((n = 105)\). The Pearson correlation coefficient calculated by SPSS is 0.693.

Norunaluwar et al, 2017
Our recent project of G6PD kit evaluation
We evaluated this method and established the normal range for G6PD activity and cut-off points for G6PD deficiency and compared the findings with OSSMRD2000.

- normal term neonates
  - carestart vs carestart 1 vs OSSMRD2000
- paediatric age 1 month – 12 years
  - carestart 1 vs OSSMRD2000
- adults
  - carestart 1 vs OSSMRD2000
  - Venepuncture vs finger-prick
All selected cord blood samples in EDTA tube were measured for G6PD activity by OSMMR2000D G6PD assay method first, then

- For the Combo kit (Carestart 1), 5μL of blood sample was applied onto the G6PD test strip and 7 μL onto the Hb strip and measurements were done simultaneously on G6PD Biosensor Combo: G6PD enzyme activity was automatically calculated.

- For the single kit, Hb measurements were done on Sysmex XN3000; while G6PD enzyme activity was measured using similar G6PD test strip on G6PD Biosensor Single: G6PD enzyme activity was then calculated manually (U/gHb

The enzyme activities measured by each method were then compared with OSMMR2000D G6PD assay method.

In this study, we used 10% of mean normal G6PD activity as cut-off point for severe deficiency.
### G6PD variants can be classified into three categories

<table>
<thead>
<tr>
<th>Class I Mutations</th>
<th>Class II Mutations</th>
<th>Class III</th>
<th>Class IV</th>
<th>Class V</th>
</tr>
</thead>
<tbody>
<tr>
<td>severe deficiency with no or minimal detected enzyme activity</td>
<td>severe deficiency with 1% to 10% residual enzyme activity</td>
<td>moderate deficiency with 10% to 60% residual enzyme activity, RBCs contain unstable G6PD enzyme, but normal activity in younger RBCs and reticulocytes</td>
<td>Normal enzyme activity at 60% to 150%</td>
<td>increased enzyme activity at &gt;150%</td>
</tr>
<tr>
<td>often associated with chronic non-spherocytic anemia (occurs even in absence of oxidative stress)</td>
<td>G6PD enzyme shows normal stability but, very low activity in all RBCs</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**WHO Working Group, 1989**
# G6PD enzymatic activity – G6PD assay kit by Biosensor Carestart: range for Newborn

<table>
<thead>
<tr>
<th></th>
<th>Carestart</th>
<th>Carestart 1</th>
<th>OSMMR2000D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean G6PD activity (U/gHb)</td>
<td>9.4</td>
<td>9.3</td>
<td>13.42</td>
</tr>
<tr>
<td>Upper limit of severe def (U/gHb) (10% of mean value)</td>
<td>0.9</td>
<td>0.9</td>
<td>1.342</td>
</tr>
<tr>
<td>Upper limit of moderate def (U/gHb) (60% of mean value)</td>
<td>5.6</td>
<td>5.6</td>
<td>8.052</td>
</tr>
</tbody>
</table>

*Darnina et al, 2017*
G6PD enzymatic activity – G6PD assay kit by Biosensor Carestart: range for Newborn

Correlation study between Carestart Biosensor and Carestart Biosensor 1 with OSMMR2000D showed strong Spearman correlation coefficient; 0.783 for the single kit and 0.769 for the combo kit.

Both POCT kits also showed strong agreement with OSMMR2000D G6PD assay method, as illustrated by kappa value of 0.805 and 0.795 for single and combo kit respectively.

Darnina et al, 2017
Peripheral blood samples in EDTA tube from 63 paediatrics and 62 adults with normal G6PD activity measured by Hb-normalization OSMMR2000D G6PD assay kit were used to evaluate Carestart™ Biosensor 1 kit (POCT assay kit).

All adult samples were collected from staffs of Haematology Unit with additional blood samples from finger prick.
<table>
<thead>
<tr>
<th></th>
<th>Carestart™ Biosensor 1</th>
<th>OSSMR 2000D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paediatric</td>
<td>Adult</td>
</tr>
<tr>
<td></td>
<td>Venepuncture</td>
<td>Finger-Prick</td>
</tr>
<tr>
<td>Mean G6PD activity (U/gHb)</td>
<td>6.8</td>
<td>6.3</td>
</tr>
<tr>
<td>Upper limit of severe def (10% of mean value U/g Hb)</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Upper limit of moderate def (60% of mean value U/g Hb)</td>
<td>4.1</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Normal G6PD activity by both methods and cut-off points for severe and moderate deficiency.  

Najiah et al, 2017
Correlation between G6PD activities in peripheral blood of adults taken by finger-prick and venepuncture. The Pearson correlation coefficient is 0.728.

Najiah et al, 2017
Correlation between G6PD activities in paediatric blood samples (normal and deficient samples) measured by OSMMR 2000D and Carestart™ Biosensor 1. The Pearson correlation coefficient is 0.7899.

Najiah et al, 2017
Challenges in assessing the performance of currently available tests for G6PD deficiency:

- Poor harmonization in evaluation criteria
- Problem of defining normal G6PD activity; described as mean or median activity for a given population.
- Problem in defining severe G6PD deficiencies – most studies took G6PD activity <0.1% - 15% (Nicole et al, 2014), but some study, even > 20% considered to be deficient
Evaluation of tests for G6PD def

• G6PD reference normal values easily obtained – large normal samples, but not for deficient samples.
• Smaller samples – prevalence of severely def individuals may skew the population normal value.
• Recent approach suggested by Domingo et al, 2013 – to establish a normal G6PD reference value for a population – by excluding < 10% median activity.
Histogram showing the G6PD enzymes level in cord blood of 94 normal term neonates – after excluded outliers –

- My own study – for determination of cut-off point for G6PD def, only selected normal G6PD activity samples

Azma et al, 2010
Evaluation of tests for G6PD def

• Study done by Nicole et al, 2014 compared between two G6PD assay tests (OSMMR-D vs binaxNOW) showed:
  – Clustered severe deficient samples (ours also showed very low SD)
  – Overall correlation was moderate – Pearson correlation coefficient of 0.7585 (ours – 0.693:Mindray, ~ 0.7 – 0.8: Carestart))
  – Absolute activity values were significantly different for the same samples – mean G6PD activity OSMMR vs binaxNOW: 10.33 vs 7.17 U/g Hb
    • OSSMR 18.59 vs Mindray 25.22 U/g Hb
    • OSSMR 13.42 vs carestart 9.3 Ug Hb

#(highlights the sensitivity of enzyme activity to reactions condition as well as platforms)
Figure 2. Correlation between G6PD activities measured by Trinity and R&D quantitative tests ($N = 214$). The Pearson correlation coefficient is 0.7585. $N = 214$.

BinaxNOW vs OSMMR-D – showed moderate correlation
We selected 188 cord bloods in EDTA tubes and refrigerated at 2-8°C for seven days.

Out of 188, 32 samples were spotted on chromatography paper, air-dried and stored at room temperature.

G6PD enzyme activities were measured daily for 7 days using the OSMMR2000-D G6PD Assay Kit on both the EDTA blood and spotted blood samples.

Norunaluwar et al, 2016
Figure: Mean G6PD activity in EDTA cord blood samples from normal neonates.

The daily mean G6PD activity of newborns with normal G6PD activity showed a significant drop on the fourth day of storage.

Table: No statistically significant difference between Day 1 and Day 2, and Day 1 and Day 3.

Statistically significant difference between Day 1 and Day 4 and the subsequent days.

Norualuwar et al, 2016

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Mean of score difference (95% CI)</th>
<th>t-stats (df)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1</td>
<td></td>
<td>0.117 (0.001 - 0.225)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>15.089</td>
<td>(1.325)</td>
<td></td>
<td>2.152 (171) 0.033</td>
</tr>
<tr>
<td>Day 2</td>
<td>14.972</td>
<td>(1.364)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>14.901</td>
<td>(1.425)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>14.729</td>
<td>(1.441)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td>14.387</td>
<td>(1.529)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td>14.348</td>
<td>(1.436)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>14.247</td>
<td>(1.536)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P-value <0.005 shows there was significant difference in mean G6PD activity.
• Figure 2: Mean G6PD activity in EDTA cord blood samples with deficient G6PD Activity (n=16)
• No statistically significant differences between Day 1 and Day 2 (p=0.327).
• Statistically significant differences between Day 1 and Day 3, and the subsequent days (p<0.005).

Norunaluwar et al, 2016
Comparison between stored blood from EDTA tube and stored blood spots on chromatography paper (n=32).

Significant reduction in enzyme activity as early as the second day of storage.

Statistically significant difference in the mean G6PD activity between day 1 and day 2 for stored blood spots (13.9 U/g Hb vs 12.02 U/g Hb, p < 0.005).

Norunaluwar et al, 2016
Following that study, now UKMMC no longer used any spotted, air-dried cord blood on chromatography samples even for FST analysis (to avoid false negative results).

- All samples are collected using EDTA tubes only.
- Samples in EDTA tubes – analysed within 3 days.
Near patient testing

• Most of our Orang Asli in Malaysia stay at very remote area and high malaria endemic.

• High incidence of G6PD deficiency among orang Asli – Temiar sub-ethnic of Senoi, incidence in males 29.8%, in females 29.8%

• In Malaysia, infection by Plasmodium vivax is the highest (50.2% from 4725 reported cases (MOH, 2012))
Near patient testing

- Reliable G6PD screening method very important before any anti-malaria treatment can be given (Antimalarial 8-aminoquinoline (primaquine) induces acute haemolytic crisis in patient with G6PD deficiency).

- Due to this remote location, blood sampling by EDTA tubes are not suitable since temperature of storage during transportation may affect enzyme level.

- Even spotted of blood on chromatography paper will also affect enzyme level.
Near patient testing. Why it is important in Malaysia?

- Near patient testing is very suitable to detect G6PD def among our Orang Asli, where it gives immediate result for decision to institute anti-malaria treatment.
  - Able to measure enzyme level: thus accurately diagnosed both moderate and severe G6PD deficiency compare to FST
  - Bancone G, et al, 2015 recommended 30% and above as the cut-off for primaquine treatment to be considered as safe.
  - Reduce burden of samples transportation

- Also suitable for our District hospital at remote area (esp Sabah and Sarawak) with small number of births daily
- or in any Haematology laboratories in Malaysia
  - No need QC maintenance.
  - No need to reevaluate when using new batch of reagents (less maintainance)
  - Immediate results
IMPORTANT REFERENCES


IMPORTANT REFERENCES

• Darnina Abd Jalil, Azlin Ithnin, Raja Zahratul Azma, Mimi Azura Aziz, Cheah Foo Choe, Nazarudin Safian, Najiah Ajlaa Ayub, Malisa Mohd Yusoff, and Ainoon Othman. Evaluation of POCT; Carestart biosensor; single kit and Carestart Biosensor 1; Combo Kit vs the standard reference method (OSMMR2000D) in the quantification of G6PD enzyme level. Abstract of the 14th Annual Scientific Meeting, Malaysian Society of Haematology, 2017.


• Endom I, Danny KXR, Azma RZ, Hamidah NH, Ainoon O, Aizzat Naeeim N, Hanisah S, Zafirah Z, Nurul Izzati Atiqi S, Siti Noor Baya MN. G6PD deficiency among Senoi Orang Asli’s Children: Recommendation for a mandatory population screening. Proceeding of the 8th Malaysia-Indonesia-Brunei Medical Sciences Conferences, Faculty of Medicine, Universitas Indonesia 2013.


Thank you!