GSP® Neonatal G6PD kit (3310-0010) is a new, fully automated G6PD (glucose-6-phosphate dehydrogenase) enzyme activity assay to be used with the GSP® system. Optimized for the quantitative determination of G6PD activity in blood specimens dried on filter paper, the kit is an effective aid in screening newborns for G6PD deficiency (Figure 1.). To determine analytical performance of the kit, verification studies were performed at PerkinElmer, Turku, Finland and an evaluation study was conducted at a customer site to ensure that the new product meets the user requirements and performs in its intended use.

**Key advantages over manual methods:**

- Fully automated assay
- Reliable sample results even with floating disks
- 24 hrs valid calibration curve
- Elution control for missing or poorly eluted samples
- All reagents and QC material are bar-coded
- On-board stability 14 days
- Simultaneous screening of any other GSP analytes in any preferred order

Figure 1. Workflow comparison of manual G6PD assay and automated GSP Neonatal G6PD kit
Complete selection of reagents
One kit contains reagents for running 12 plates (Figure 2.)
• GSP G6PD Calibrators – 7 dried blood spot sets
• GSP G6PD Controls – 4 dried blood spot sets
• GSP G6PD Assay Buffer – 3 bottles, ready-for-use
• GSP G6PD Substrate 1 (NADP) – 3 vials, lyophilized
• GSP G6PD Substrate 2 (G6P) – 3 vials, ready-for-use

Validated assay method
The GSP Neonatal G6PD assay is based on the same enzymatic reaction as the manual, PerkinElmer Neonatal G6PD (ND-1000) assay (Figure 3.). G6PD enzyme present in the sample catalyzes the conversion of glucose-6-phosphate to 6-phosphogluconate while reducing NADP+ to NADPH. To determine G6PD activity levels, the fluorescence of NADPH is measured using an excitation wavelength of 340 nm and emission wavelength of 460 nm.

Novel technology to measure floating disks
With the GSP Neonatal G6PD assay, a novel measurement technology enabled the measurement of wells with floating disks. The accuracy of GSP Neonatal G6PD kit to measure floating disks was verified by repeated measurements of both floating and non-floating dried blood samples of varying G6PD activity level.

Specimen stability
The influence of storage time, temperature, and humidity on G6PD activity was studied using several dried whole blood spot samples.

Precision, lower limits of detection and linearity
The precision of the assay was determined in accordance with CLSI document EP05-A2 [1] using human whole blood spot samples, 3 kit lots and 3 GSP instruments. The study was performed with 54 plates measured over 20 working days, each plate had 4 replicates per sample. The total number of measurements was 216 per sample. An analysis of variance approach was used to calculate the variance components. The analytical limits were determined in accordance with CLSI document EP17-A2 [2] and linearity was determined in accordance with CLSI document EP06-A [3].

Independent evaluation study
An evaluation study was conducted at the Newborn Screening Center, National Institutes of Health of the Philippines, Manila. The study aimed:
• To determine the usability of the GSP Neonatal G6PD kit
• To produce normal newborn population distribution data for the GSP Neonatal G6PD kit

The study included 2,075 routine newborn screening specimens and 38 archived confirmed G6PD deficient specimens. G6PD activity levels were measured using GSP Neonatal G6PD kit (3310-0010) and a manual method (ND-1000). Patient mean, median, minimum, maximum and cut-off values corresponding to 10th, 5th and 3.25th percentile were calculated for the GSP Neonatal G6PD kit.
**Results**

**Assay Performance**

**Measurement of floating disks**

The ability of the GSP Neonatal G6PD kit to measure wells containing floating disks was verified by repeated measurements of both floating and non-floating samples with varying G6PD activity levels. The measured sample activity was not significantly affected by floating disks (Figure 5.).

![Figure 5. Effect of floating disks on GSP G6PD activity measurements](image)

**GSP Neonatal G6PD kit calibration curve**

The calibration unit was defined as units per decilitre (U/dL) of whole blood. One unit of G6PD enzyme catalyses the formation of 1 µmol of NADPH in 1 minute at 25 °C and pH 8.1. The kit calibrators cover the clinically relevant activity range and the reagents include controls for low (15 U/dL) and high (50 U/dL) G6PD activity. The calibration curve is valid for 24 hours. A typical calibration curve is shown in Figure 6.

![Figure 6. A typical calibration curve for GSP Neonatal G6PD assay](image)

**Table 1. Variation of GSP Neonatal G6PD kit using one calibration curve valid for 24 hours.**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>N</th>
<th>MEAN G6PD ACTIVITY (U/dL)</th>
<th>WITHIN RUN VARIATION</th>
<th>WITHIN LOT VARIATION</th>
<th>TOTAL VARIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>216</td>
<td>3.0</td>
<td>CV%</td>
<td>CV%</td>
<td>CV%</td>
</tr>
<tr>
<td>2</td>
<td>216</td>
<td>9.7</td>
<td>7.0</td>
<td>10.9</td>
<td>12.2</td>
</tr>
<tr>
<td>3</td>
<td>216</td>
<td>12.9</td>
<td>2.9</td>
<td>3.3</td>
<td>4.2</td>
</tr>
<tr>
<td>4</td>
<td>216</td>
<td>18.0</td>
<td>3.3</td>
<td>3.9</td>
<td>4.8</td>
</tr>
<tr>
<td>5</td>
<td>216</td>
<td>24.9</td>
<td>4.1</td>
<td>4.8</td>
<td>4.9</td>
</tr>
<tr>
<td>6</td>
<td>216</td>
<td>37.6</td>
<td>4.7</td>
<td>4.8</td>
<td>4.8</td>
</tr>
<tr>
<td>7</td>
<td>216</td>
<td>94.1</td>
<td>7.8</td>
<td>8.1</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Analytical limits, measuring range and linearity**

Analytical limits, measuring range and linearity of the GSP Neonatal G6PD kit are summarized in Table 2. The Limit of Blank (LoB) is defined as the 95th percentile of a distribution of blank samples (n=150), the Limit of Detection (LoD) is based on 863 determinations of low level samples and the Limit of Quantitation (LoQ) is defined as the lowest activity with a total CV equal to or less than 20%.

**Specimen stability**

Storage of specimens at elevated temperature and humidity conditions significantly decreases the observed G6PD activity. At +35 °C with high humidity, 50 % of the G6PD activity may be lost in just 1 day (Figure 7.).

![Figure 7. The G6PD activity (% of reference without storage, 52 U/dL) during storage at different temperatures and humidity conditions.](image)

**Precision**

The within-run, within-lot and total variation results for the GSP Neonatal G6PD kit are shown in Table 1.
**Evaluation Study**

**Frequency distribution for newborn screening specimens**

The frequency distributions of the newborn screening specimens for G6PD activity are shown in Figure 8. Confirmed G6PD deficient specimens are shown with red bars and light blue bars indicate presumed normal specimens, which include non-confirmed G6PD deficient specimens.

Descriptive statistics of the routine newborn screening specimens results for G6PD activity are shown in Table 3 and the results for the retrospective G6PD deficient specimens in Table 4.

**Screening performance**

The new GSP Neonatal G6PD assay was compared to the manual Neonatal G6PD kit (ND-1000) by measuring G6PD activity of neonatal samples with both methods. The distribution is visualized in Figure 9. Red circles represent confirmed G6PD specimens and light blue circles presumed normal specimens (which include non-confirmed G6PD deficient specimens). By using lower 5th percentile cut-off value, 20.5 U/dL, GSP Neonatal G6PD kit found all the confirmed G6PD deficient specimens and missed none.

![Figure 8. Frequency distribution of the newborn screening specimens for G6PD activity. Red bars represent confirmed G6PD deficient specimens (including both retrospective and confirmed G6PD deficient routine specimens) and light blue bars presumed normal (include non-confirmed G6PD deficient specimens).](image)

![Figure 9. Scatter plot of GSP Neonatal G6PD results as a function of manual G6PD activity. Red circles represent confirmed G6PD deficient samples.](image)

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>SAMPLE SIZE</th>
<th>MEAN (U/dL)</th>
<th>MEDIAN (U/dL)</th>
<th>MIN (U/dL)</th>
<th>MAX (U/dL)</th>
<th>3.25%</th>
<th>5.00%</th>
<th>10.00%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine*</td>
<td>2075</td>
<td>46.4</td>
<td>47.7</td>
<td>0.1</td>
<td>141.7</td>
<td>16.1</td>
<td>20.5</td>
<td>26.4</td>
</tr>
<tr>
<td>* Include also confirmed and non-confirmed G6PD deficient specimens.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Range, mean and median G6PD activity values with lower percentiles for the routine specimens measured with GSP Neonatal G6PD kit

<table>
<thead>
<tr>
<th>SAMPLE TYPE</th>
<th>SAMPLE SIZE</th>
<th>MEAN (U/dL)</th>
<th>MEDIAN (U/dL)</th>
<th>MIN (U/dL)</th>
<th>MAX (U/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrospective confirmed G6PD deficient</td>
<td>38</td>
<td>7.7</td>
<td>7.8</td>
<td>0.3</td>
<td>17.9</td>
</tr>
</tbody>
</table>

Table 4. Range, mean and median G6PD activity values for the retrospective, confirmed G6PD deficient specimens measured with GSP Neonatal G6PD kit
Conclusions

- Reliable sample results obtainable even with floating disks
- The GSP Neonatal G6PD kit demonstrates good analytical performance
- Storage of specimens with elevated temperature and humidity increases the risk of false positive results
- All retrospective confirmed G6PD deficient samples were identified by the GSP Neonatal G6PD kit
- The fully automated GSP Neonatal G6PD assay saves time and money in a newborn screening laboratory.

Acknowledgements

Dr. Carmencita D. Padilla, Newborn Screening Center, Institute of Human Genetics, NIH University of the Philippines Manila

References

