**Reagents**

<table>
<thead>
<tr>
<th></th>
<th>R1</th>
<th>Antibody Buffer</th>
<th>Anti-Phenobarbital antibody. Sodium azide 0.09% w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R2</td>
<td>Latex Reagent</td>
<td>Latex. Sodium azide 0.09% w/v</td>
</tr>
</tbody>
</table>

**Calibration**

The use of Therapeutic Drug Calibrator is recommended. We recommend a Multi Point calibration every 7 days, each change of reagent bottle/lot or according to quality control procedures.

**Preparation**

Reagents: Ready for use.

Calibrator/Control: Lyophilized. Reconstitute with the indicated volume on the respective instructions of use.

Calibration Curve: Must be used the Spinreact Therapeutic Drug Calibrator.

**Storage and Stability**

R1. Antibody Buffer

Stable up to expiry date when stored at 2-8°C. Do not freeze. Before use, reagents should be gently swirled to dislodge bubbles and ensure homogeneity.

R2. Latex Reagent

Stable up to expiry date when stored at 2-8°C. Do not freeze. Before use, reagents should be gently swirled to dislodge bubbles and ensure homogeneity.

**Additional equipment**

- Spectrophotometer or colorimeter measuring at 700 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

**Samples**

Samples should be stored at 2-8°C prior to testing for up to three days. Samples intended for assay after three days from collection should be frozen at -20°C until use. Any additional clotting or precipitation that occurs due to the freeze/thaw treatment should be removed by centrifugation prior to analysing the Phenobarbital concentration of that sample.

**Procedure**

1. Bring the reagents and the photometer (cuvette holder) to 37°C.
2. Assay conditions:
   - Wavelength: 700 nm
   - Temperature: 37°C
   - Cuvette light path: 1 cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:
   - Reagent R1: 990 µL
   - Sample or Calibrator: 9 µL
   - Reagent R2: 360 µL

5. Mix and read the absorbance (A1) after the R2 addition.
6. Incubate at 37°C and read the absorbance (A2) exactly 4 minutes after the R2 addition.

**Calculations**

Calculate the absorbance difference (A2-A1) of each point of the calibration curve and plot the values obtained against the Phenobarbital concentration of each calibrator dilution. Phenobarbital concentration in the sample is calculated by interpolation of its (A2-A1) in the calibration curve.

**Quality control**

Control sera are recommended to monitor the performance of manual and automated assay procedures. Therapeutic Drug Controls kit Ref.: 939550 (3 levels). Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

**Reference values**

Adults: 15 – 40 µg/mL

Children: 15 – 30 µg/mL

These values are for orientation purpose; each laboratory should establish its own reference range.

**Performance Characteristics**

**Assay range:** The range of this assay is approximately 2.90 - 87.7 µg/mL depending on the concentration range of the Phenobarbital calibrators in use. Samples with concentrations in excess of the highest calibrator should be diluted with the 0 µg/mL calibrator, re-assay and multiply the result with the appropriate dilution factor.

**Precision:**

<table>
<thead>
<tr>
<th></th>
<th>Intra-assay (n=20)</th>
<th>Inter-assay (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µg/mL)</td>
<td>8.44</td>
<td>13.56</td>
</tr>
<tr>
<td>SD</td>
<td>0.323</td>
<td>0.84</td>
</tr>
<tr>
<td>CV (%)</td>
<td>3.80</td>
<td>6.22</td>
</tr>
</tbody>
</table>

**Accuracy:** Results obtained using reagents (y) did not show systematic differences when compared with other commercial reagent (x). The results obtained using 40 samples spanning the range 5.66 to 55.34 µg/mL were the following:

- Correlation coefficient (r): 0.9955
- Regression equation: y = 1.11x + 1.6103

The results of the performance characteristics depend on the analyzer used.

**Interferences**

The following analytes were tested up to the following levels and were found not to interfere:

- Intralipid®
- Bilirubin: 800 mg/dl
- Triglyceride: 1000 mg/dl
- Haemoglobin: 1000 mg/dl

**Notes**

1. In order to avoid contamination it is recommended to use disposable material.
2. M.H. has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

**Bibliography**