Quantitative determination of Phenobarbital

IVD
Store at 2-8°C

PRINCIPLE OF THE METHOD
This is a quantitative turbidimetric test for the measurement of Phenobarbital in human serum. Latex particles coated with Phenobarbital are agglutinated when mixed with Phenobarbital Antibody solution. When a sample containing Phenobarbital is used, the agglutination reaction is partially inhibited, slowing the process of agglutination. The agglutination causes an absorbance change, inversely dependent upon the Phenobarbital contents of the patient sample.

CLINICAL SIGNIFICANCE
Phenobarbital is an antiepileptic and sedative-hypnotic drug. The results of this test are used to control Phenobarbital levels and to assure an appropriate therapy.

REAGENTS

<table>
<thead>
<tr>
<th>R 1</th>
<th>Anti-Phenobarbital antibody. Sodium azide 0.09% w/v</th>
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<tbody>
<tr>
<td>R2</td>
<td>Latex. Sodium azide 0.09% w/v</td>
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CALIBRATION
The use of Therapeutic Drug Calibrator is recommended. We recommend a Multi Point calibration each 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 between 2-8°C prior to testing for up to three days. Samples intended for assaying 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.:399550 (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>Mean (µg/mL)</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-assay (n=20)</td>
<td>9.84</td>
<td>0.323</td>
</tr>
<tr>
<td>Inter-assay (n=20)</td>
<td>8.44</td>
<td>0.286</td>
</tr>
</tbody>
</table>

Accuracy: Results obtained using reagents (y) did not show systematic differences when compared with other commercial reagent (x).

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:

<table>
<thead>
<tr>
<th></th>
<th>µg/dl</th>
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<tbody>
<tr>
<td>Intra lipid®</td>
<td>800</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>25</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>1000</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>1000</td>
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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

REF. 939040
2 x 17 mL, 2 x 6 mL