Phenytoin

Immunoassay - TDM

Quantitative determination of Phenytoin
IVD
Store at 2-8ºC

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

CLINICAL SIGNIFICANCE
Phenytoin is an antiepileptic drug that is used to control focal and generalized motor seizures (fits, tonic-clonic).
The range of serum concentrations at which Phenytoin toxicity occurs is broad. Toxic effects, such as nystagmus and distaxia can occur at concentrations above 20 µg/mL. Ataxia and eye movement abnormalities may occur above 30 µg/mL.

REAGENTS

R 1 Antibody Buffer	Anti- Phenytoin antibody. Sodium azide 0.09% w/v
R2 Latex Reagent	Latex. Sodium azide 0.09% w/v

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 Phenytoin concentration of that sample.

PROCEDURE
1. Bring the reagents and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:
   Wavelength : 700
   Temperature : 37 ºC
   Cuvette ligth path : 1cm
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 37ºC 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 Phenytoin concentration of each calibrator dilution. Phenytoin 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. Spinreact has the 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
10 – 20 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 1.1- 40 µg/mL depending on the concentration range of the Phenytoin 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:

	Intra-assay (n=20)			Inter-assay (n=20)		
Mean (µg/mL)	4.99	15.80	25.01	4.52	14.55	21.30
SD	0.184	0.268	0.461	0.220	0.340	0.468
CV (%)	3.68	1.69	1.84	4.88	2.34	2.20

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 2.37 to 39.3 µg/mL were the following:
Correlation coefficient (r): 0.9962.
Regression equation: y= 1.0515x + 1.635
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®	800 mg/dl
Bilirubin	25 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

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2. *Tisdale J.E., Tsuyuki R. T., Oles K. S., Penry J.K.:Relationship between serum concentrations and dose of valproic acid during monotherapy in adult outpatients. Therapeutic Drug Monitoring 1992, **14**; 416-423.*
3. *Chadwick D.W.: Valproate monotherapy in the Management of generalized and partial seizures. Epilepsia 1987, **78 (supplement 2):** 512-7.*
4. *Chadwick D.W.: Concentration effect relationships of Valproic Acid. Clinical Pharmacokinetics 1985, **10**; 155-163.*
5. *Newman, D.J., Henneeberry H., Price C.P., "Particle Enhanced Light Scattering Immunoassay", Ann. Clin. Biochem., **29**: 22-42 (1992)*

REF. 939050 | Cont. | 2 x 17 mL, 2 x 6 mL

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