DXC (PHY) PHENYTOIN (DILANTIN)

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PURPOSE

To provide instruction for the quantitative determination of phenytoin (Dilantin) on the DXC 600/800.

PRINCIPLE

PHY reagent, when used in conjunction with UniCel® DxC 600/800 System(s) and SYNCHRON® Systems Drug Calibrator 1 set, is intended for quantitative determination of total Phenytoin concentration in human serum or plasma.

BACKGROUND

Clinical Significance

Phenytoin is indicated for the treatment of grand mal and cortical focal seizures and temporal lobe epilepsy. Phenytoin therapy is monitored for suspected inadequate dose or toxicity.

Methodology

PHY reagent is used to measure analyte concentration by a particle enhanced turbidimetric inhibition immunoassay method. Particle-bound drug (PBD) binds to the analyte specific antibody (Ab) resulting in the formation of insoluble aggregates causing light scatter. Non-particle-bound analyte in the patient sample competes with the PBD for the antibody binding sites, inhibiting the formation of insoluble aggregates. The rate and amount of particle aggregation is inversely proportional to the concentration of analyte in the sample. The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 101 parts reagent. The system monitors the aggregate formation by measuring the change in absorbance at 340 nanometers. This change in absorbance is inversely proportional to the concentration of phenytoin in the sample and is used by the System to calculate and express the phenytoin concentration based upon a multi-point calibration curve.

RELATED DOCUMENTS

R-PO-CH0810	Quality Control Program General Laboratory
R-PO-CH0809	Quality Control Westgard Rules Statistics
R-PR-AD0540	Specimen Rejection/Cancellation Protocol
J-F-CH0820	DXC 800 Controls
J-F-CH0826	DXC 800 Calibrators
J-F-CH1940	DXC 800 Analytical Measurement Range
M-F-CH0820	Chemistry Controls
M-F-CH0826	Chemistry Calibrators
M-F-CH1940	DXC 600 Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum or plasma are the preferred specimens. Acceptable anticoagulants are listed in the PROCEDURAL NOTES section of this chemistry information sheet. Whole blood or urine are not recommended for use as a sample.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

Sample Type	Volume	Sample Stability
Plasma/Serum	0.5mL	• 8 hours at 18-26° **C** • 48 hours at 2-8° **C** • After 48 hours, freeze at -15 to -20° C

Criteria for Unacceptable Specimens

See Specimen Rejection/Cancellation Protocol

Sample Volume

A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS

Contents

Each kit contains the following items:
Two PHY Reagent Cartridges, kit # 469188 (2 x 100 tests)

Volume per Test	
Sample Volume	3 µL
Total Reagent Volume	302 µL
Cartridge Volumes	A 230 µL B 40 µL C 32 uL

Reactive Ingredients	
Phenytoin Particle Reagent	4.8 mL
Monoclonal anti-Phenytoin Antibodies (mouse)	7.0 mL
Phenytoin Reaction Buffer	80.0 mL

Also non-reactive chemicals necessary for optimal system performance.

Reagent Preparation

No preparation is required. Do not mix.

Acceptable Reagent Performance

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within your facility`s acceptance criteria.

Reagent Storage and Stability

PHY Reagent when stored unopened at +2°C to +8°C, will remain stable until the expiration date printed on the cartridge label. Once opened, the reagent is stable for 42 days at +2°C to +8°C. Do not use beyond the manufacturer's expiration date. DO NOT FREEZE. Do not expose reagent to temperatures above +35°C or to direct sunlight.

CALIBRATION

Calibrator Required

SYNCHRON® Systems Drug Calibrator 1 set

Calibrator Preparation

No preparation is required.

Calibrator Storage and Stability

SYNCHRON® Systems Drug Calibrator 1 set is stable until the expiration date printed on the calibrator bottle if stored capped in the original container at +2°C to +8°C. Do not use beyond the manufacturer's expiration date.

Calibration Information

1. The system must have a valid calibration curve in memory before control or patient samples can be run.

2. Under typical operating conditions the PHY reagent cartridge must be calibrated every 14 days and also with certain parts replacements or maintenance procedures, as defined in the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual. This assay has within-lot calibration available. Refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual for information on this feature.

3. For detailed calibration instructions, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

Traceability

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

See Related Documents J-F-CH0820 DXC 800 Controls & M-F-CH0820 Chemistry Controls

STEPS

1. If necessary, load the reagent onto the system.

2. After reagent load is completed, calibration may be required.

3 Program controls for analysis.

4. After loading controls onto the system, follow the protocols for system operation. To load samples manually refer to the FHS DXC Series Manual Sample Programming procedure. For detailed testing procedures, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

CALCULATIONS

SYNCHRON® System(s) perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

ANTICOAGULANT TEST RESULTS

If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method based on a study of 20 healthy volunteers:

Anticoagulant	Level Tested for In Vitro Interference	Average Plasma-Serum Bias (µg/mL)
Lithium Heparin	14 Units/mL	NSI[b]
Sodium Heparin	14 Units/mL	NSI

PERFORMANCE CHARACTERISTICS

Reference Range

Therapeutic	10.0 – 20.0 µg/mL
Critical (Toxic)	>25.0 µg/mL

For Critical Value reporting protocol, refer to FHS Critical Policy

Analytic Range

The SYNCHRON® System(s) method for the determination of this analyte provides the following analytical ranges:

Sample Type	Conventional Units
Serum or Plasma	2.5 – 40.0 µg/mL

Samples reported out as "SUPPRESSED" due to RXN ERROR should be reanalyzed.

Reporting results outside of analytical range

Lower limit of range: serum / plasma	2.5 µg/mL	Results less than 2.5; Report as <2.5 ug/mL
Upper limit of range: serum / plasma	40.0 µg/mL	Results >40.0, should be diluted with with 0.9% saline, reanalyzed and dilution factor applied. The maximum allowable dilution is X2. Results >80.0 should be reported as >80.0 µg/mL.

Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for PHY determination is 2.5 µg/mL.

LIMITATIONS

Falsely elevated phenytoin concentrations have been observed in patients with renal deficiency or failure.

Interferences

1. The following substances were tested for interference with this methodology:

Substance	Source	Level Tested	Observed Effect
Hemoglobin	RBC hemolysate	500 mg/dL INDEX 10	NSI[d]
Bilirubin	Porcine	30 mg/dL INDEX 20	NSI
Rheumatoid Factor	Human	300 IU/mL	NSI
Lipemia	Human	500 mg/dL INDEX 10	NSI
Paraprotein (IgM)	Human	500 mg/dL	NSI

2. Refer to References (9,10,11,12) for other interferences caused by drugs, disease and preanalytical variables.

3. For assays employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Human anti-mouse antibodies may be present in samples from patients who have received immunotherapy or diagnostic procedures utilizing monoclonal antibodies or in individuals who have been regularly exposed to animals.[13,14] Additionally, other heterophile antibodies, such as human anti-goat antibodies may be present in patient samples. Interpretation of results should be done in the context of the overall clinical presentation of the patient, including symptoms, clinical history, data from additional tests and other appropriate information.

ADDITIONAL INFORMATION

For more detailed information on UniCel DxC Systems, refer to the appropriate system manual.

REFERENCES

1. Newman, D. J., Henneberry, H., Price, C. P., "Particle Enhanced Light Scattering Immunoassay", *Ann. Clin . Biochem.*, 29:22 42 (1992).

2. Tietz, N. W., "Specimen Collection and Processing; Sources of Biological Variation", *Textbook of Clinical Chemistry*, 2nd Edition, W. B. Saunders, Philadelphia, PA (1994).
3. National Committee for Clinical Laboratory Standards, *Procedures for the Handling and Processing of Blood Specimens*, Approved Guideline, NCCLS publication H18-A, Villanova, PA (1990).
4. CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, U.S. Government Printing Office, Washington, D.C. (1984).
5. Burtis, C. A., Ashwood, E. R., *Tietz Textbook of Clinical Chemistry*, 2nd Edition, W. B. Saunders, Philadelphia, PA (1994).
6. National Committee for Clinical Laboratory Standards, *How to Define, Determine, and Utilize Reference Intervals in the Clinical Laboratory*, Approved Guideline, NCCLS publication C28-A, Villanova, PA (1995).
7. Tietz, N. W., ed., *Fundamentals of Clinical Chemistry*, 3rd Edition, W. B. Saunders, Philadelphia, PA (1987).
8. Henry, J. B., *Clinical Diagnosis and Management by Laboratory Methods*, 18th Edition, W. B. Saunders Company, Philadelphia, PA (1991).
9. Young, D. S., *Effects of Drugs on Clinical Laboratory Tests*, 4th Edition, AACC Press, Washington, D. C. (1995).
10. Friedman, R. B., Young, D. S., *Effects of Disease on Clinical Laboratory Tests*, 3rd Edition, AACC Press, Washington, D.C. (1997).
11. Young, D. S., *Effects of Preanalytical Variables on Clinical Laboratory Tests*, 2nd Edition, AACC Press, Washington, D. C. (1997).
12. Legatt, D., Rostron, P. A., Simpson, I., Higgins, T., "Measurement of Phenytoin Concentrations in Uremic Patients Using Immunoassay Methods: An Old Problem Revisited", *Journal of Clinical Immunoassay* , Vol. 16, No. 1, (Spring 1993).
13. Bjerner, J., et al., "Immunometric Assay Interference: Incidence and Prevention", *Clin. Chem.* 48:613 621 (2002).
14. Kricka, L. J., "Interferences in Immunoassays-Still a Threat", Clin. Chem., 46:1037 1038 (2000).
15. National Committee for Clinical Laboratory Standards, *Method Comparison and Bias Estimation Using Patient Samples*, Approved Guideline, NCCLS publication EP9-A, Villanova, PA (1995).
16. National Committee for Clinical Laboratory Standards, *Precision Performance of Clinical Chemistry Devices*, Tentative Guideline, 2nd Edition, NCCLS publication EP5-T2, Villanova, PA (1992).

DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
Standardized formatting using small tables. Added Maximum dilution. Incorporated Updated Index information.			
Committee Approval Date	☒ Date: 1/8/2015 ☐ NA – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Katie Wilkinson, MD 7/30/15