BECKMAN COULTER CE

SYNCHRON System(s)
Chemistry Information Sheet

PHE
Phenobarbital

REF 469785

For *In Vitro* Diagnostic Use

Rx Only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

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

CLINICAL SIGNIFICANCE

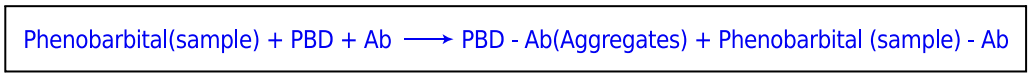
Phenobarbital is indicated for the treatment of status epilepticus, febrile seizures and seizure disorders (grand mal and psychomotor), except absence (petit mal) seizures. Phenobarbital therapy is monitored for suspected inadequate dose or toxicity.

METHODOLOGY

PHE reagent is used to measure the PHE concentration by a particle enhanced turbidimetric inhibition immunoassay method.[1] A particle-bound drug (PBD) binds to PHE specific antibody (Ab) resulting in the formation of insoluble aggregates causing light scatter. Non-particle-bound PHE in the patient sample competes with the PBD for the antibody binding sites, inhibiting formation of insoluble aggregates. The rate and amount of particle aggregation is inversely proportional to the concentration of PHE 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 98 parts reagent. The system monitors aggregate formation by measuring the change in absorbance at 340 nanometers. This change in absorbance is inversely proportional to the concentration of PHE in the sample and is used by the System to calculate and express the PHE concentration based upon a multi-point calibration curve.

CHEMICAL REACTION SCHEME



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SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.[2] 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.[3]
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.[3]

Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE VOLUME

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:

Two PHE Reagent Cartridges (2 x 100 tests)

VOLUMES PER TEST

Sample Volume	3 µL
Total Reagent Volume	295 µL
Cartridge Volumes	
A	210 µL
B	55 µL
C	30 µL

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

Phenobarbital Particle Reagent	4.5 mL
Monoclonal anti-phenobarbital Antibody (mouse)	8.2 mL
Phenobarbital Reaction Buffer	32.0 mL

Also non-reactive chemicals necessary for optimal system performance.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

Avoid skin contact with reagent. Use water to wash reagent from skin.

GHS HAZARD CLASSIFICATION

Phenobarbital Reagent (Compartment A)

DANGER

H318	Causes serious eye damage.
H412	Harmful to aquatic life with long lasting effects.
P273	Avoid release to the environment.
P280	Wear protective gloves, protective clothing and eye/face protection.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor/physician.
	Polyoxyethylated Octyl Phenol 1 - 10%

SDS Safety Data Sheet is available at techdocs.beckmancoulter.com

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON® Systems Drug Calibrator 1
At least two levels of control material
Saline

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

PHE 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 unless the expiration date is exceeded. DO NOT FREEZE. Do not expose reagent to temperatures above +35°C or to direct sunlight.

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON® Systems Drug Calibrator 1

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

SYNCHRON® Systems Drug Calibrator 1 is stable until the expiration date printed on the calibrator bottle if capped and stored in the original container at +2°C to +8°C.

⚠ CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.[4]

Calibrator storage location:

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 PHE 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.
3. This assay has within-lot calibration available. 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

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

Table 1.0 Quality Control Material

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

1. If necessary, load the reagent onto the system.
2. After reagent load is completed, calibration may be required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operations.

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

CALCULATIONS

The SYNCHRON System(s) performs 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.

REPORTING RESULTS

Equivalency between the SYNCHRON CX, SYNCHRON LX, and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

REFERENCE INTERVALS

Therapeutic PHE concentrations vary significantly, depending upon the individual. The lower limit for one patient may be ineffective in another, while the upper limit may prove toxic in a third. The physician should determine the appropriate therapeutic interval for each patient. The reference intervals listed below were taken from literature.[5]

Table 2.0 Reference intervals

INTERVALS	SAMPLE TYPE	THERAPEUTIC INTERVAL		TOXIC INTERVAL	
		CONVENTIONAL UNITS (µg/mL)	S.I. UNITS (µmol/L)	CONVENTIONAL UNITS (µg/mL)	S.I. UNITS (µmol/L)
Literature	Serum/Plasma	15–40	65 – 170	Slowness, ataxia, nystagmus:	
				35 – 80	151 – 345
				Coma with reflexes	
				65 – 117	280 – 504
				Coma without reflexes	
				> 100	> 430

INTERVALS	SAMPLE TYPE	THERAPEUTIC INTERVAL		TOXIC INTERVAL	
		CONVENTIONAL UNITS (µg/mL)	S.I. UNITS (µmol/L)	CONVENTIONAL UNITS (µg/mL)	S.I. UNITS (µmol/L)
Laboratory	Serum/Plasma				

Refer to References (6,7,8) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

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:

Table 3.0 Acceptable Anticoagulants[a]

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

a Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.

b NSI = No Significant Interference (within ± 2.0 µg/mL or 8%).

LIMITATIONS

None identified

INTERFERENCES

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

Table 4.0 Interferences[a]

SUBSTANCE	SOURCE	LEVEL	OBSERVED EFFECT[b]
Bilirubin (unconjugated)	Bovine	30 mg/dL	NSI[c]
Hemoglobin	RBC hemolysate	500 mg/dL	NSI
Lipemia	Intralipid[d]	500 mg/dL	NSI

a Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.
b Plus (+) or minus (-) signs in this column signify positive or negative interference.
c NSI = No Significant Interference (within ±2.0 µg/mL or 8%).
d Intralipid is a registered trademark of KabiVitrum, Inc., Clayton, NC 27250.

2. Refer to References (9,10,11) 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.[12,13] 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.

SPECIFICITY

The following list of substances were added at the concentration listed to separate aliquots of a serum pool containing 21.0 µg/mL phenobarbital. In most cases the value shown approximates maximum physiological concentrations. The recovered values were subtracted from the serum pool value. If the results were within ± 2X of the within-run precision specifications there was no significant interference. If the recovered results were more than ± 2X of the within-run precision specifications the difference is listed under observed effect.

Table 5.0 Specificity[a]

SUBSTANCE	CONCENTRATION (µg/mL)	OBSERVED RECOVERY (µg/mL)	OBSERVED EFFECT (µg/mL)
Amobarbital	4	23.4	+2.4
Carbamazepine	25	22.0	NSI[b]
Carb-[10,11]-Epoxide	20	21.3	NSI
Chlordiazepoxide	20	20.7	NSI
Chlorpromazine	20	21.4	NSI
Diazepam	10	20.4	NSI
DL-glutethimide	25	22.0	NSI
Ethosuximide	500	20.4	NSI
5-Ethyl-5-p-hydroxyphenyl-barbituric acid	100	20.6	NSI

Table 5.0 Specificity, Continued

SUBSTANCE	CONCENTRATION (µg/mL)	OBSERVED RECOVERY (µg/mL)	OBSERVED EFFECT (µg/mL)
5-(p-hydroxyphenyl)-5-phenylhydantoin (HPPH)	15	21.1	NSI
Mephenytoin	50	20.3	NSI
Mephobarbital	5	26.1	+5.1
Methsuximide	100	21.1	NSI
2-Ethyl-2-phenylmalonamide (PEMA)	50	21.6	NSI
Pentobarbital	2.5	21.2	NSI
Phenytoin	75	20.6	NSI
Primidone	100	21.0	NSI
Secobarbital	15	21.1	NSI
Valproic Acid	200	22.6	NSI
	100	21.2	NSI

a Data shown was collected using SYNCHRON CX Systems. Equivalency between SYNCHRON LX Systems has been established by Deming regression analysis to SYNCHRON CX Systems.

b NSI = No Significant Interference (within ±2.0 µg/mL or 8%).

PERFORMANCE CHARACTERISTICS

ANALYTIC RANGE

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

Table 6.0 Analytical Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Serum or Plasma	5.0 – 80.0 µg/mL	21.5 – 345.0 µmol/L

Samples with concentrations outside the analytical range will be reported as "<5.0 µg/mL" ("<21.5 µmol/L") or ">80.0 µg/mL" (">345.0 µmol/L").

Samples reported out as greater than the analytical range may be confirmed by diluting with saline and reanalyzing. The appropriate dilution factor should be applied to the reported result.

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 7.0 Reportable Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

SENSITIVITY

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for PHE determination is 5.0 µg/mL (21.5 µmol/L).

EQUIVALENCY

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

Serum or plasma (in the range of 5.2 to 45.5 µg/mL):

Y (SYNCHRON LX Systems)	= 0.976X + 0.50
N	= 85
MEAN (SYNCHRON LX Systems)	= 18.13
MEAN (SYNCHRON CX7 DELTA)	= 18.07
CORRELATION COEFFICIENT (r)	= 0.992

Refer to References (14) for guidelines on performing equivalency testing.

PRECISION

A properly operating SYNCHRON System(s) should exhibit imprecision values less than or equal to the maximum performance limits in the table below. Maximum performance limits were derived by an examination of the imprecision of various methods, proficiency test summaries, and literature sources.

Table 8.0 Maximum Performance Limits

TYPE OF PRECISION	SAMPLE TYPE	1 SD		CHANGEOVER VALUE[a]		% CV
		µg/mL	µmol/L	µg/mL	µmol/L	
Within-run	Serum/Plasma	1.0	4.3	25.0	107.8	4.0
Total	Serum/Plasma	1.5	6.5	25.0	107.8	6.0

a When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

Comparative performance data for a SYNCHRON LX® System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.[15] Each laboratory should characterize their own instrument performance for comparison purposes.

Table 9.0 NCCLS EP5-T2 Precision Estimate Method

TYPE OF IMPRECISION	SAMPLE TYPE		No. Systems	No. Data Points[a]	Test Mean Value (µg/mL)	EP5-T2 Calculated Point Estimates	
						SD	%CV
Within-run	Serum	Control 1	1	80	9.32	0.22	2.3
	Serum	Control 2	1	80	65.86	1.42	2.2
Total	Serum	Control 1	1	80	9.32	0.33	3.5
	Serum	Control 2	1	80	65.86	2.31	3.5

a The point estimate is based on the pooled data from one system, run for twenty days, two runs per day, two observations per run on an instrument operated and maintained according to the manufacturer's instructions.

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX® System and are not intended to represent the performance specifications for this reagent.

ADDITIONAL INFORMATION

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

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REVISION HISTORY

Revision AG

Corrected the translation of days in the Calibration Stability section of the Greek translation.

Revision AH

Updated corporate address; updated OSHA precaution and removed EDTA as an Acceptable Anticoagulant claim.

Revision AJ

Added Revision History.

Revision AK

Added new language requirement: Czech, and Korean.

Revision AL

Removed references to CX and LX systems as they are discontinued effective 12/2013.

Added Beckman Coulter trademark statement and disclaimer.

Revision AM

Added GHS Classification information

REFERENCES

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