510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K210858

B Applicant

Ortho-Clinical Diagnostics, Inc.

C Proprietary and Established Names

VITROS Chemistry Products PHBR Slides

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
DLZ	Class II	21 CFR 862.3660 - Phenobarbital Test System	TX - Clinical Toxicology

II Submission/Device Overview:

A Purpose for Submission:
New device

B Measurand:
Phenobarbital

C Type of Test:
Quantitative enzyme immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
For in vitro diagnostic and laboratory professional use.

VITROS Chemistry Products PHBR Slides quantitatively measure phenobarbital (PHBR) concentration in serum and plasma (lithium heparin) using the automated VITROS 5600 Integrated System.

Measurements obtained by this device are used as an aid in the diagnosis and treatment of phenobarbital use or overdose and in monitoring levels of phenobarbital to help ensure appropriate therapy.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:
Automated VITROS 5600 Integrated System

IV Device/System Characteristics:

A Device Description:
The VITROS PHBR Slide is a multilayered, analytical element coated on a polyester support. The phenobarbital assay is based on an enzymatic heterogeneous, competitive immunoassay format. Immobilized anti-phenobarbital antibody and phenobarbital-peroxidase conjugate are present in the spreading layer.

B Principle of Operation:
The determination of Phenobarbital concentrations is performed using the VITROS Chemistry Products PHBR Slides in conjunction with the VITROS Chemistry Products Calibrator Kit 9 on the VITROS 5600 Integrated System.
The phenobarbital assay is based on an enzymatic heterogeneous, competitive immunoassay format. Immobilized anti-phenobarbital antibody and phenobarbital-peroxidase conjugate are present in the spreading layer. Phenobarbital in the sample competes with the phenobarbital-peroxidase conjugate for a limited number of antibody binding sites. The subsequent addition of the VITROS Immuno-Wash Fluid to the slide removes unbound phenobarbital-peroxidase conjugate from the read area, while also providing a substrate for the enzyme mediated oxidation of leuco dye. The rate of dye formation, as monitored by reflectance spectrophotometry, is inversely proportional to the phenobarbital concentration in the sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):
ARCHITECH iPhenobarbital Assay

B Predicate 510(k) Number(s):
K081231

C Comparison with Predicate(s):

Device & Predicate Device(s):	K210858	K081231
Device Trade Name	VITROS Chemistry Products PHBR Slides	ARCHITECT iPhenobarbital Assay
General Device Characteristic Similarities		
Intended Use/Indications For Use	For the quantitative measurement of phenobarbital in human serum or plasma	Same
General Device Characteristic Differences		
Assay Range	3.0 – 80.0 µg/mL	1.10 – 80.0 µg/mL

VI Standards/Guidance Documents Referenced:

CLSI EP25-A: *Evaluation of Stability of In Vitro Diagnostic Reagents*

CLSI EP37: *Supplemental Tables for Interference Testing in Clinical Chemistry, 1st Edition*

CLSI EP05-A3: *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline*

CLSI EP06: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, 2nd Edition*CLSIEP07*: Interference Testing in Clinical Chemistry, 3rd Edition*

CLSI EP35: *Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures, 1st Edition*

CLSI EP17-A2: *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures*

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:
   Precision was evaluated with patient pools and quality control materials following CLSI EP05-A3guideline. The precision performance of VITROS Chemistry Products PHBR Slides

was evaluated internally, on the VITROS 5600 Integrated System, with three VITROS Chemistry Systems PHBR Slides reagent lots. Three serum based controls and 4 serum samples spanning the analytical measuring interval were evaluated for performance. Each precision sample was assayed in replicates of two, 2 runs per day for 22 days for a total of 88 replicates per sample for each lot.
The results of the precision studies are shown in the table below:

Slide Lot	Conc. µg/mL	Repeatability		Within-Laboratory	
		SD	% CV	SD	%CV
1	5.0	0.20	3.9%	0.25	5.0%
	9.1	0.27	2.9%	0.33	3.6%
	11.0	0.30	2.7%	0.44	4.0%
	23.0	0.50	2.2%	0.65	2.8%
	25.1	0.59	2.4%	0.81	3.2%
	38.1	0.79	2.1%	1.09	2.9%
	59.3	1.50	2.5%	2.11	3.6%
2	4.6	0.20	4.3%	0.33	7.1%
	8.8	0.33	3.8%	0.59	6.7%
	11.2	0.36	3.2%	0.58	5.2%
	25.5	0.70	2.7%	0.96	3.8%
	28.1	0.59	2.1%	0.93	3.3%
	41.2	0.77	1.9%	1.01	2.4%
	58.7	1.28	2.2%	1.58	2.7%
3	5.4	0.21	4.0%	0.28	5.1%
	9.1	0.33	3.7%	0.43	4.7%
	11.1	0.36	3.3%	0.58	5.3%
	24.4	0.69	2.8%	1.01	4.1%
	25.9	0.72	2.8%	1.20	4.6%
	39.0	1.26	3.2%	1.64	4.2%
	59.3	2.82	4.8%	4.19	7.1%

2. Linearity:
Linearity studies were performed according to CLSI EP06-A guideline. A series of fourteen proportionally related admixtures of low and high concentration samples were tested in duplicates. The sample concentrations ranged from 0.5 to 83.5 µg/mL. The results of the linearity studies support that the assay is linear over the claimed measuring range of 3.0 – 80.0 µg/mL.

Sample Dilution Studies:
Serum and plasma samples with values greater than 80.0 µg/mL may be diluted with one part sample and one part diluent. Dilution studies were performed to determine the sample recovery after a 1:2 dilution is performed automatically by the VITROS 5600 Integrated System. A total of 12 serum and 12 plasma (lithium heparin) samples with phenobarbital concentrations between 80.0 to 160.0 µg/mL were diluted 1:2 on-analyzer using the VITROS Chemistry Products Specialty Diluent and run in triplicate. The average percent differences for the diluted sample versus the expected concentration was within 10%. The dilution study

results support the sponsor's labeling claims that samples with phenobarbital concentrations above the 80.0 µg/mL may be diluted 1:2 on- analyzer to obtain results up to 160.0 µg/mL.

3. Analytical Specificity/Interference:

The VITROS Chemistry Products PHBR Slides method was screened for interfering substances at phenobarbital concentrations of approximately 15 and 50 µg/mL following CLSI EP07 and EP37 guidelines. Each test and control sample were run in replicates of 6 on 3 reagent lots. The sponsor defined significant interference as a bias > 1.8 µg/mL at a phenobarbital concentration of approximately 15 µg/mL or a bias > 5.2 µg/mL at a phenobarbital concentration of approximately 50 µg/mL.

The following substances did not interfere with the VITROS Chemistry Products PHBR Slides at the concentrations shown below.

Substance	**Concentration**	**Substance**	**Concentration**
Acetaminophen	15.6 mg/dL	Glucose	1000 mg/dL
Amitriptyline	0.048 mg/dL	Glutethimide	3.6 mg/dL
Amoxicillin	5.4 mg/dL	Glyburide	72 µg/dL
Ascorbic acid	5.25 mg/dL	HPPH	14.3 mg/dL
Atenolol	0.9 mg/dL	Hydrochlorothiazide	0.11 mg/dL
Bilirubin, unconjugated	40 mg/dL	Ibuprofen	21.9 mg/dL
Caffeine	10.8 mg/dL	Intralipid	2000 mg/dL
Carbamazepine	4.5 mg/dL	Lamotrigine	4.5 mg/dL
Cephalexin	12.6 mg/dL	Levetiracetam	27 mg/dL
Chlordiazepoxide	0.69 mg/dL	Levodopa	0.75 mg/dL
Chlorpromazine	0.33 mg/dL	Lithium	6.25 mg/dL
Cholesterol	400 mg/dL	Lithium Heparin	330 units/dL
Cimetidine	3.0 mg/dL	Lorazepam	72 µg/dL
Ciprofloxacin	1.2 mg/dL	Methsuximide	12 mg/dL
Clonazepam	30 µg/dL	N-acetylcysteine	15 mg/dL
Clorazepate	0.78 mg/dL	Naproxen	36 mg/dL
Codeine	0.14 mg/dL	Nifedipine	59 µg/dL
Creatinine	15 mg/dL	Nitrazepam	31 µg/dL
Dextromethorphan	1.56 µg/dL	Oxazepam	0.43 mg/dL
Diazepam	3.0 mg/dL	Phenylbutazone	32.1 mg/dL
Digoxin	3.9 µg/dL	Prednisone	10 µg/dL
Diltiazem	90 µg/dL	Pseudoephedrine	0.33 mg/dL
Diphenhydramine	77.4 µg/dL	Ranitidine	1.05 mg/dL
Dipyrone	5.1 mg/dL	Salicylic acid	2.86 mg/dL
Dopamine	62.2 µg/dL	Sodium bromide	35.5 mg/dL
Enalapril	82.1 µg/dL	Theophylline	6.0 mg/dL
Ethosuximide	30 mg/dL	Tolazamide	4.5 mg/dL
Furosemide	1.59 mg/dL	Triglycerides	1500 mg/dL
Gapapentin	8.2 mg/dL	Verapamil	0.16 mg/dL
Gentamicin	3.0 mg/dL	Warfarin	7.5 mg/dL

Substance	Concentration	Substance	Concentration
Gentisic acid	1.5 mg/dL		

The nine (9) substances listed in the tables below, when tested at the concentrations indicated, caused significant biases.

Interferent	PHBR Conc. (µg/mL)	Interferent Concentration	Bias (µg/mL)
Conjugated bilirubin	15	13.7 mg/dL	2.1
Ethamsylate		4.5 mg/dL	3.4
Hemoglobin		300 mg/dL	-2.3
Mephobarbital		0.79 mg/dL	2.6
Thiamylal		7.4 mg/dL	3.1
Thiopental		2.5 mg/dL	4.2
Total protein		13.0 g/dL	-2.8
Valproic acid		18.4 mg/dL	2.3

Interferent	PHBR Conc. (µg/mL)	Interferent Concentration	Bias (µg/mL)
Conjugated Bilirubin	50	27.4 mg/dL	6.3
Ethamsylate		3.0 mg/dL	7.2
Ethanol		6.0 mg/mL	8.1
Hemoglobin		225 mg/dL	-10.8
Mephobarbital		0.79 mg/dL	11.0
Thiamylal		7.4 mg/dL	9.1
Thiopental		10.1 mg/dL	9.1
Total protein		11.0 g/dL	-8.7
Valproic acid		36.7 mg/dL	7.1

Cross-reactivity: The cross-reactivity of the VITROS Chemistry Products PHBR Slides method was evaluated by adding the following substances to serum samples containing phenobarbital concentrations of approximately 15 and 50 µg/mL.
Cross-reactivity was expressed as the mean result obtained for the cross-reactant sample divided by the cross-reactant concentration in percentage terms.
Results of cross-reactivity testing are summarized below:

Test Substance	Conc. (µg/mL)	Mean Control Sample Result (µg/mL)	Mean Cross-reactant Sample Result (µg/mL)	% Cross-reactivity
Amobarbital	36 µg/mL	17	18	2.20%
		64	65	2.50%

Test Substance	Conc. (µg/mL)	Mean Control Sample Result (µg/mL)	Mean Cross-reactant Sample Result (µg/mL)	% Cross-reactivity
Aprobarbital	50 µg/mL	21	22	1.10%
		70	70	0.00%
Barbital	203 µg/mL	16	16	-0.10%
		58	57	-0.40%
Butabarbital	30 µg/mL	19	19	0.50%
		62	61	-3.80%
Butalbital	20 µg/mL	15	16	0.70%
		57	59	6.10%
Hexobarbital	9.5 µg/mL	15	15	2.10%
		56	55	-10.20%
Mephobarbital	10.5 µg/mL	17	24	77.40%
		53	61	84.00%
Pentobarbital	126 µg/mL	17	17	0.10%
		56	57	0.70%
Phenylethyl-malonamide (PEMA)	108 µg/mL	18	17	-0.10%
		54	54	0.30%
Phenytoin	60 µg/mL	16	17	1.70%
		56	58	2.50%
Primidone	57 µg/mL	15	15	0.60%
		54	55	2.20%

4. Assay Reportable Range:
   The claimed assay range is 3.0 – 80.0 µg/mL.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   The VITROS Chemistry Products Calibrator Kit 9 for phenobarbital are traceable to the Certified NIST (National Institute of Standards and Technology) Reference Material, SRM® (Standard Reference Material) 900.

6. Detection Limit:
   The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined for the VITROS Chemistry Products PHBR Slides assay based upon recommendations in CLSI EP17 guideline.

   The LoB and LoD studies were conducted using three VITROS Chemistry Products PHBR Slides reagent lots. The LoB testing consisted of 15 replicates of four human serum blank pools for 5 days. The LoD testing consisted of 5 replicates of four human serum samples spiked with low phenobarbital concentrations for 5 days. The LoQ testing consisted of 15 replicates of four low phenobarbital concentration serum sample pools for 3 days. The LoQ was defined as the highest phenobarbital concentration having a total allowable error of ≤1.5 µg/mL.

The LoB, LoD, and LoQ for the VITROS Chemistry Products PHBR Slides assay were determined to be 0.6 µg/mL, 1.3 µg/mL, and 3.0 µg/mL respectively.

7. Assay Cut-Off:
Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Method comparison studies were conducted by testing 142 serum samples using the VITROS Chemistry Products PHBR Slides (candidate device) and the ARCHITECT iPhenobarbital Assay (predicate device). The phenobarbital concentrations in the samples ranged from 5.59 to >80 µg/mL, as determined by the predicate device. Passing-Bablok regression analysis was performed to determine the correlation.

Regression Analysis Summary table is shown below:

Regression Analysis Summary					
N	**Slope**	**Correlation Coefficient**	**Range of Samples µg/mL**	**Intercept µg/mL**	**Sy.x**
142	0.92	0.983	5.6 - 76.1	-2.0	2.6

2. Matrix Comparison:

Matrix comparison testing followed recommendations per CLSI EP35 guideline. A total of fifty-four matched samples were tested, which included fifty-one native samples and three samples that were spiked with phenobarbital to create high concentration samples. The following matrices were evaluated: serum (reference matrix), lithium heparin plasma, serum drawn using a serum separator tube (SST), and lithium heparin plasma drawn using a plasma separator tube (PST). Samples from each matrix were tested in duplicate using three reagent lots on one VITROS 5600 Integrated System.

The regression analysis results for each specimen matrix and collection device are shown in the table below:

Slide Lot	Matrix/ Collection Device	n	Slope	Intercept	Correlation Coefficient
Lot 1	Lithium heparin Plasma	51	1.00	0.41	0.990
	Serum Separator SST	51	1.00	-0.09	0.994
	Plasma Separator PST	50	0.99	-0.11	0.992
Lot 2	Lithium heparin Plasma	51	1.03	-0.10	0.992
	Serum Separator SST	51	0.97	0.31	0.994
	Plasma Separator PST	50	0.99	0.14	0.992

Slide Lot	Matrix/ Collection Device	n	Slope	Intercept	Correlation Coefficient
Lot 3	Lithium heparin Plasma	52	1.02	-0.09	0.992
	Serum Separator SST	52	0.99	0.15	0.995
	Plasma Separator PST	51	0.98	-0.05	0.993

The results of the matrix comparison studies support the sponsor's claim that serum and lithium heparin plasma are suitable specimen matrices for use with the assay, including samples collected using serum separator (SST) and lithium heparin plasma separator tubes (PST).

C Clinical Studies:

1. Clinical Sensitivity:
   Not applicable.

2. Clinical Specificity:
   Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   Not applicable.

D Clinical Cut-Off:
Not applicable.

E Expected Values/Reference Range:
The sponsor has stated the reference interval is based on literature reports[1]:

	Conventional Units (µg/mL)	SI Units (µmol/L)	Alternate Units (mg/mL)
Therapeutic Range	10.0-40.0	65-172	10.0-40.0

Each laboratory should confirm the validity of these intervals for the population it serves.

[1]*Patsalos PN, et al. Antiepileptic drugs—best practice guidelines for therapeutic drug monitoring: A position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. Epilepsia, 49(7):1239-1276, 2008*.

VIII Proposed Labeling:
The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.