510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k042520

B. Purpose for Submission:

New Device

C. Measurand:

Caffeine

D. Type of Test:

Quantitative

E. Applicant:

Ortho-Clinical Diagnostics, Inc.

F. Proprietary and Established Names:

VITROS Chemistry Products CAFFN Reagent Kit

VITROS Chemistry Products TDM Performance Verifier I, II, and III

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.3800 Theophylline Test System

   21 CFR 862.3280 Drug Mixture Control Material

2. Classification:

   21 CFR 862.3800 Theophylline Test System, Class II.

   21 CFR 862.3280 Controls, Class I, reserved.

3. Product code:

   KLS

   DIF

4. Panel:

   91 (Toxicology), and 91 (Toxicology)

H. Intended Use:

1. Intended use(s):

   See Indications for Use below.

2. Indication(s) for use:

   For *in vitro* diagnostic use only. VITROS Chemistry Products CAFFN Reagent Kit is used on the VITROS 5,1 FS Chemistry System to quantitatively measure caffeine (CAFFN) concentration in human serum and plasma of subjects undergoing therapy with caffeine, especially for cases of neonatal apnea.

   For in vitro diagnostic use only. VITROS TDM Performance Verifier is an assayed control used to monitor performance of ACET, CRBM, DGXN, PHYT, and CAFFN on VITROS Chemistry Systems.

3. Special conditions for use statement(s):

   For Prescription Use Only.

4. Special instrument requirements:

   VITROS 5,1 FS Chemistry System (previously cleared K031924)

I. Device Description:

The VITROS Chemistry Products CAFFN Reagent Kit is a three component dual chamber device.

Component one consists of lyophilized reagent 1 and reagent 2, six levels of calibrators and buffer concentrate. Reagent 1 in the VITROS CAFFN Reagent Kit is sheep polyclonal antibodies reactive to caffeine, glucose-6-phosphate (Na-G6P), Nicotinamide Adenine Dinucleotide (NAD) and rabbit serum albumin. Reagent 2 in the VITROS CAFFN Reagent Kit is caffeine labeled with glucose-6-phosphate dehydrogenase (G6P-DH), glucose-6-phosphate (Na-G6P) and rabbit serum albumin.

Component two is the VITROS CAFFN Verifiers I, II and III, which are a set of 3 verifiers (6 vials- 2 mL each) and are required for the VITROS CAFFN Reagent Kit.

Component three is the Diluent Pack 3 is a dual chamber that is used to dilute samples and uses processed water and a specialty diluent prepared from processed human serum.

The VITROS CAFFN Kit and the Diluent Pack 3 was tested using FDA approved methods and was found nonreactive for hepatitis B surface antigen (HBsAg), antibody to HCV and antibody to HIV. This information is indicated in the labeling.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   SYVA Emit Caffeine Assay

   VITROS Chemistry Products TDM Performance Verifiers, I, II, and III

2. Predicate 510(k) number(s):

   SYVA Emit Caffeine Assay k853872

   VITROS Chemistry Products TDM Performance Verifiers – k982649

3. Comparison with predicate:

   VITROS Chemistry Products CAFFN Reagent Kit

Similarities		
Item	Device	Predicate
Range	1- 30 µg/mL	1- 30 µg/mL
Calibrator	6 levels included	6 levels included
Stability	Opened: 12 weeks	Opened: 12 weeks
Storage	2-8°C	2-8°C

Differences		
Item	Device	Predicate
Intended Use	For *in vitro* diagnostic use only. VITROS Chemistry Products CAFFN Reagent Kit is used on the VITROS 5,1 FS Chemistry System to quantitatively measure caffeine (CAFFN) concentration in human serum and plasma of subjects undergoing therapy with caffeine, especially for cases of neonatal apnea.	Quantitative measurement of caffeine as a metabolite.
Sample	Serum and Plasma	Serum
Instrumentation	VITROS 5,1 FS Chemistry System	SYVA-30R Biochemical System

VITROS Chemistry Products TDM Performance Verifiers

Similarities		
Item	Device	Predicate
Intended Use	For in vitro diagnostic use only. VITROS TDM Performance Verifier is an assayed control used to monitor performance of ACET, CRBM, DGXN, PHYT, and CAFFN on VITROS Chemistry Systems.	For in vitro diagnostic use only. VITROS TDM Performance Verifier is an assayed control use to monitor performance on VITROS Chemistry System.
Matrix	Serum	Serum
Levels	I, II and III	I, II and III

Differences		
Item	Device	Predicate
Instrumentation	VITROS 5,1 FS Chemistry System	SYVA-30R Biochemical System
Constituents	Addition of Caffeine(CAFFN), Digoxin (DGXN) and Phenytoin(PHYT) to the already cleared Acetaminophen (ACET), Carbamazepine(CRBM) and Phenobarbital (PHBR).	Acetaminophen (ACET), Carbamazepine (CRBM), Phenobarbital (PHBR).

K. Standard/Guidance Document Referenced (if applicable):

NCCLS document EP9-A. Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline- Second Edition.

NCCLS document EP5-A. Method Comparison of Precision Performance of Clinical Chemistry Devices; Approved Guideline.

NCCLS document EP7-A. Interference Testing in Clinical Chemistry; Approved Guideline.

NCCLS document EP6-A. Evaluation of the Linearity of Quantitative Measurement Procedure: A Statistical Approach; Approved Guideline.

L. Test Principle:

The VITROS Chemistry Products CAFFN Reagent Kit is a two-step reaction that quantitatively measures caffeine. Caffeine labeled with a glucose-6-phosphate dehydrogenase (G6P-DH) competes with caffeine in a sample for antibody binding sites. G6P-DH conversion of Nicotinamide Adenine Dinucleotide (NAD+) to NADH and the sample is read spectrophometrically at 340 nm. The absorbance change is proportional to the caffeine concentration in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Two lots of the VITROS Chemistry Products CAFFN Reagent were tested in duplicate on two separate VITROS 5,1 FS Systems. Same day runs were separated by a two hour minimum. Each run assayed three control sera in duplicate for mean concentrations of 2.62, 12.55 and 21.30 μg/mL. Mean values for the samples that were tested for within-day, within-lab precision, within-lab %CV and the number of observations are shown in the table below.

Mean Concentration	Within Day SD	Within Lab SD	Within Lab %CV	# of Observation
2.62 μg/mL	0.092	0.171	6.5 %	88
12.55 μg/mL	0.291	0.553	4.4 %	88
21.30 μg/mL	0.536	0.957	4.5 %	84

Water from the VITROS Chemistry Products FS Diluent Pack 3 (Specialty Diluent/Water) was tested for dilution ability and accuracy with two VITROS

Chemistry Products Reagent lots. Ten aliquots of normal human serum samples spiked with caffeine stock solutions ranging from 22.5 to 30 μg/mL. The samples were diluted 1:2 and 1:4 with water from the Diluent Pack 3. The samples were tested in triplicate and a mean was calculated for the neat samples and the diluted samples. For Lot 1, the percent recovery for the 1:2 and 1:4 diluted samples was 91.5% and 86.5 % respectively. For Lot 2, the recovery for the 1:2 and 1:4 diluted samples was 94.5% and 89.8% respectively.

b. Linearity/assay reportable range:

Two caffeine spiked pools of serum high pool (55 μg/mL) and a low pool (near zero μg/mL) were mixed to create 15 additional pools of intermediate concentrations. Five determinations for each fluid level and three determinations of VITROS Chemistry Products TDM Performance Verifiers were conducted with two lots of CAFFN Reagents on two VITROS 5,1 FS Chemistry Systems. A plot of the observed results (between 0.7-32 μg/mL) versus the expected results yielded an equation of Y=0.4345X-0.2018 and an R^2 of 0.9931 between 0.7-32 μg/mL. Acceptable linearity was defined as when the bias between predicted and calculated caffeine concentrations were within predetermined acceptance limits.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The VITROS Chemistry Products CAFFN Reagent Kit Calibrators are traceable to a USP Caffeine reference standard (catalogue # 1085003). Primary calibrators are prepared by the manufacturer and the six levels are confirmed by GC/MS or HPLC methods. The sponsor confirms the calibrator values using another test system. One hundred clinical serum samples are assigned values from this run. The serum samples are then used to calibrate the VITROS test system, and to assign values to the sponsor's master lot of calibrators. This procedure ensures that matrix effects associated with both methods are taken into account in the value transfer process.

The VITROS Chemistry Products were found to have a stability of 12 weeks when open and/or reconstituted at 2-8 °C. On board stability of the VITROS Chemistry Products CAFFN Reagent Kit is 12 weeks if the system is turned on and 20 minutes if the system is turned off. This claim is similar to the predicate's.

The VITROS TDM Performance Caffeine (CAFFN), Digoxin (DGXN) and Phenytoin (PHYT) Verifiers are included in this submission. The VITROS TDM Performance Verifiers are prepared gravimetrically from bovine serum, therapeutic drugs, inorganic salts and preservatives. The VITROS TDM Performance Verifiers have an open vial stability of 7 days when stored at 2-8 °C. The VITROS TDM Performance Verifiers Control values are

assigned using two instruments and include two calibrations, multiple samples of each reagent lot and 32 replicates of the performance verifier. An allowable range is then applied around these reagent lot-specific target values to establish upper and lower control limits. The allowable range reflects estimates of expected within-lab and lab-to-lab variability of properly operating VITROS 5,1 FS Chemistry Systems. A range of means was determined on 5 VITROS 5,1 FS Chemistry System in different laboratories for 10 days, 2 runs per day and 2 replicates per run for each performance verifier. A pooled SD was determined using the total SD's from each site to give a +/- range as 3 times the pooled SD.

d. Detection limit:

The lower limit of detection was studied using 4 determinations with 2 reagent lots and 2 calibrator lots on two VITROS 5,1 FS Chemistry Systems. Ten serum samples from normal adult human donors that contained little or no caffeine were analyzed in triplicates using a low level calibrator, a serum base pool and saline. It was determined that the low level calibrator had no caffeine. Serum caffeine concentrations from 3 of the 10 donors were greater than 0.5 μg/mL and were excluded from the study. The lower limit of detection was calculated using the following equation:

$$LLD=3.3*(\text{Calibration error Variance } (SD)^2 + \text{Pooled Replicate Variance } (SD)^2)^{1/2}.$$

The lower limit of detection ranged from 0.14 μg/mL to 0.15 μg/mL and supports that claim of 0.2 μg/mL.

e. Analytical specificity:

The substances listed in the table below at the concentrations shown, were tested according to NCCLS Protocol EP7-A with VITROS CAFFN Reagent and a serum pool at a caffeine concentration of 7 μg/mL (with the exception of Bilirubin, Hemoglobin and Intralipid). An off the clot human serum pool spiked with caffeine to create caffeine concentrations of 0.6 μg/mL was spiked with a caffeine stock of 10,000 μg/mL to create 2 base pools of caffeine concentrations of approximately 5 and 20 μg/mL. These two pools were used in testing bilirubin, hemoglobin and Intralipid. The results are shown in the table below:

Compound	Concentration	
Bilirubin	60 mg/dL	1026 μmol/L
8-Chlorotheophylline	100 μg/mL	466 μmol/L
1,3-Dimethyluric Acid	100 μg/mL	510 μol/L
Dyphylline	100 μg/mL	393 μmol/L
Hemoglobin	1000 mg/dL	10 g/L

Compound	Concentration	
Hypoxanthine	100 μg/mL	735 μmol/L
Intralipid	1000 mg/dL	10 g/L
3-Isobutyl 1-	50 μg/mL	225 μmol/L
1-Methyluric Acid	100 μg/mL	549 μmol/L
3-Methyluric Acid	100 μg/mL	549 μmol/L
1-Methylxanthine	100 μg/mL	602 μmol/L
3-Methylxanthine	100 μg/mL	602 μmol/L
7-Methylxanthine	100 μg/mL	602 μmol/L
Paraxanthine	4 μg/mL	22 μmol/L
Phenobarbital	100 μg/mL	431 μmol/L
Theobromine	30 μg/mL	167 μmol/L
Theophylline	50 μg/mL	278 μmol/L
1,3,7-Trimethyluric Acid	30 μg/mL	143 μmol/L
Urea	200 μg/mL	1.2 μmol/L
Xanthine	100 μg/mL	657 μmol/L

Interference was determined by calculating bias using the following equation:

Bias= Mean conc. of test substance pool – Mean conc. of Control pool.

The samples tested for Bilirubin, Hemoglobin and Intralipid interference with a serum pool at a caffeine concentration of approximately 5 and 20 μg/mL and was found not to interfere (bias <3.91 μg/mL). All other substances listed in the table above were tested with VITROS CAFFN Reagent Kit and a serum pool with caffeine concentration of 7 μg/mL and was found not to interfere (bias of <1.92 μg/mL).

f. Assay cut-off:

N/A

2. Comparison studies:

a. Method comparison with predicate device:

The sponsor states that studies were based on NCCLS Guideline EP9-A2 *Method Comparison and Bias Estimation Using Patient Samples*.

A total of 122 samples, sixty-six human serum and fifty-six plasma samples (110 neonatal and 20 adult samples), were assayed in triplicate with two lots of both the VITROS CAFFN assay and the Syva EMIT Caffeine assay. Caffeine concentrations for both the neonatal and adult samples ranged from

1.09 to 23.45 μg/mL. A plot of the correlation between the VITROS CAFFN Assay and the Syva EMIT Caffeine assay and yielded a least squares linear regression of Y= 0.9995X -0.04 μg/mL and a correlation coefficient of 0.989.

b. Matrix comparison:

To determine which specimen types are suitable for analysis with the assay, the sponsor conducted a study involving varios specimens from differenct Becton Dickinson tubes.

Ten whole blood samples were collected in red top serum tubes and seperated. The serum was spiked with caffeine and transferred to various tube types. The samples were studied to determine the appropriate specimen types and proper fill levels for the VITROS CAFFN Assay. The specimens were tested in triplicate with two lots of the VITROS CAFFN Reagent kit. The following table shows specimen types and fill levels for the specimens examined.

Sample Type	**Fill Levels Collected**
Serum (Red Top)	Full
Serum Separator (SST)	Full, ½ Full
Li-Heparin Plasma	¼ Full
Li-Heparin Plasma Separator (PST)	Full
Sodium Citrate	Full
EDTA Plasma	¼ Full
Sodium Fluoride Potassium Oxalate	Full

Serum and plasma samples were evaluated by paired-difference testing. The data was tabulated and the bias between the mean value (n=3) for each test condition was compared ot the mean of the serum value. The bias was calculated as :

Bias = Test Condition Prediction – Serum Sample Value

Serum (red top) was used as the reference becasue it is the specimen matrix used to establish overall accuracy of the method.

The acceptabne criteria are based on the caffeine concentration in the serum sample :

Caffeine Concentration (μg/mL)	Bias Limits
Less than 5 μg/mL	+/- 1.160 μg/mL
Greater than 5 μg/mL	+/- 0.1661[caffeine] + 0.2540

Serum samples (red top tubes), samples collected in full serum separator tubes (SST), and tubes containing the anticoagulant lithium heparin were within the sponspors predetermined acceptance criteria for the VITROS Chemistry

Products CAFFN assay.

The sponsor reported that serum samples collected in partially filled serum separator (1/2 filled) tubes may show a positive bias ranging from 3 to 11%. Serum samples collected in tubes containing EDTA did **not** meet acceptance criteria. Although serum samples collected in tubes containing Sodium Citrate or Sodium Flouride Potassium Oxalate bias values were within were within predetermined acceptance criteria, the sponsor makes no claim for these specimen types.

3. Clinical studies:

   a. Clinical Sensitivity:

   N/A

   b. Clinical specificity:

   N/A

   c. Other clinical supportive data (when a. and b. are not applicable):

   N/A

4. Clinical cut-off:

   N/A

5. Expected values/Reference range:
   Reference ranges are presented below.

Classification	Conventional Unit (µg/mL)	SI Units (µmol/L)	Alternate Units (mg/L)
Therapeutic	8 -20	41 - 103	8 - 20
Toxic (Critical)	>50	>258	>50

These reference intervals are in published literature recommended by the National Academy of Clinical Biochemistry (NACB). Use of the same ranges is further supported by the close agreement observed in the linear regression plot from the method comparison study involving the predicate assay.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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