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

A. 510(k) Number:

k062077

B. Purpose for Submission:

Clearance of new device

C. Measurand:

Amphetamine

D. Type of Test:

Qualitative and semi-quantitative Enzyme Immunoassay

E. Applicant:

Ortho-Clinical Diagnostics, Inc.

F. Proprietary and Established Names:

VITROS Chemistry Products AMPH Reagent
VITROS Chemistry Products Calibrator Kit 26
VITROS Chemistry Products FS Calibrator 1
VITROS Chemistry Products Dat Performance Verifiers I, II, III, IV and V

G. Regulatory Information:

1. Regulation section:

   21 CFR §862.3100, Amphetamine test system
   21 CFR §862.3200, Clinical toxicology calibrators
   21 CFR §862.3280, Clinical toxicology control material

2. Classification:

   Class II, II and Class I (reserved)

3. Product code:

   DKZ, DLJ, DIF, respectively

4. Panel:

   Toxicology (91)

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 AMPH Reagent is used on VITROS 5,1 FS Chemistry Systems for the semi-quantitative and qualitative determination of amphetamines (AMPH) in human urine using a cutoff of either 500, or 1000 ng/mL. Measurements obtained with the VITRO AMPH method are used in the diagnosis and treatment of amphetamines use or overdose.

   The VITRO Chemistry Products AMPH assay is intended for use by professional laboratory personnel. It provides only a preliminary test result. A more specific alternative chemical method must be used to confirm a result obtained with this assay. Gas Chromatography/Mass Spectrometry (GC/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be applied to any drug-of-abuse test result, particularly when evaluating a preliminary positive result.

   VITROS Chemistry Products Calibrator Kit 26 is used to calibrate VITROS 5,1 FS Chemistry Systems for the qualitative and semi-quantitative measurement of drugs of abuse.

   VITROS Chemistry Products FS Calibrator 1 used in conjunction with VITROS Chemistry Products Calibrator Kits to calibrate VIROS 5,1 FS Chemistry Systems.

   VITROS Chemistry Products DAT Performance Verifiers are assayed controls used to monitor performance of urine drugs of abuse screening assays on VITROS 5,1 FS Chemistry Systems.

3. Special conditions for use statement(s):

   For use by professional laboratory personnel. For in vitro diagnostic use only.

4. Special instrument requirements:

   VITROS 5,1 FS Chemistry Systems (k031924)

I. Device Description:

The VITROS AMPH Reagent consists of a dual chambered package containing two liquid ready-to-use reagents used in a two-step reaction.

Reagent 1 consists of murine monoclonal antibodies reactive to d-amphetamine and d-methamphetamine, NAD, and Glucose-6-phosphate. Reagent 2 consists of d-amphetamine and d-methamphetamine labeled with glucose-6-phosphate dehydrogenase.

Calibrator Kit 26 is a standard that is sold separately. It is a one level of aqueous solution containing d-methamphetamine (AMPH), with a concentration of 2000 ng/mL. The standard is diluted with the FS Calibrator 1 to construct the standard curve which is used to calculate the concentration of the unknown samples.

Verifiers I, II, III, IV and V are a set of 6 assayed controls (6 vials – 20 mL each) that are run with the samples to monitor the performance of the assay. They consist of human urine which drugs of abuse, metabolites of drugs of abuse, organic salt, surfactants and preservative have been added.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Syva EMIT II Plus Amphetamines assay and Bio-Rad Liquicheck Urine Toxicology Controls

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

   k031004, k022707 respectively

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for Use	For in vitro diagnostic use only. The assay is intended for use in the qualitative and semi-quantitative analysis of amphetamines in human urine. The controls are assayed controls used to monitor the performance of Chemistry systems.	Same
Test Principle	Homogeneous enzyme immunoassay	Same
Specimen type	Human Urine	Same
Reagent format	Liquid ready-to-use	Same
Antibody source	Mouse monoclonal antibodies reactive to d-amphetamine and d-methamphetamine	Same

Differences		
Item	Device	Predicate
Calibrator levels	6 levels	Qualitative – two levels Semi-quantitative – 300 ng/mL cutoff Value – four levels, 500 and 1000 ng/mL Cutoff values – five levels
Calibrator format	Frozen liquid ready to use	Refrigerated liquid ready to use
Control levels	Five	Two
Control Analytes Control Analytes cont.	Cocaine metabolites (benzoylecgonine), benzodiazepines (lormetazepam), methadone, amphetamines (d-methamphetamine), opiates (morphine), cannabinoids (11-nor-delta-THC-9-COOH), phencyclidine and barbiturates (secobarbital).	Methamphetamine, seco-barbital, lormetazepam, tetrahydrocannabinol (THC), benzoylecgonine, ethanol, lysergic acid diethylamide (LSD), methadone, methaqualone, morphine (FREE), phencyclidine, propoxyphene, nortriptyline and addition of creatinine, pH, specific gravity.

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

CLSI EP5-A: Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline
CLSI EP7-P; Interference Testing in Clinical Chemistry; Proposed Guideline
CLSI EP6-P; Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Proposed Guideline
CLSI EP12-A; User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline
CLSI EP9-A2; Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition
CLSI EP17-A; Protocols for Demonstration, Verification and Evaluation of Limits of Detection and Quantitation; Approved Guideline

L. Test Principle:

The test is an enzyme immunoassay for use on the Vitro 5,1 FS Chemistry System. Calibrators ranging in concentration from 0 to 2000 ng/mL are run with the assay. The Vitros AMPH assay is a homogenous enzyme immunoassay technique used for the qualitative and semi-quantitative analysis of amphetamine in human urine. In the performance of the Vitros AMPH assay, samples, calibrators and controls are treated with surfactant (DAT Diluent 2) prior to addition of the reagents. The treated sample is mixed with Reagent 1 which contains antibodies reactive to d-amphetamine and d-methamphetamine, glucose-6-phosphate and nicotinamide adenine dinucleotide (NAD+). Subsequently Reagent 2 containing d-amphetamine and d-methamphetamine, labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) is added. Amphetamine in the treated sample and the d-amphetamine and d-methamphetamine labeled G6PDH compete for the antibody binding sites. Enzyme activity decreases upon binding to the antibody, so amphetamine concentration in the sample can be measured in terms of enzyme activity. Enzyme activity converts NAD+ to NADH resulting in an absorbance change that is measured spectrophotometrically at 340 nm.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   The samples used for testing were the sponsor's controls, Verifiers I, II, III, IV and V. The samples were run in duplicate, twice a day for twenty-two days using two lots and four instruments. The results are presented in the table below:

Control Mean Concentration (ng/mL)	Within Day SD	Within Lab SD	Within Lab CV%
217	14.1	33.9	15.6
370	12.1	23.6	6.4
642	12.1	25.4	4.0
784	11.8	28.1	3.6
1255	27.1	71.6	5.7

An additional study was performed using three quality control materials targeted at the assay's cutoff concentrations. The controls were run 20 replicates per day for five days using a single lot of reagent. The results are presented in the table below:

Cutoff Value (ng/mL)	Mean Measured Concentration	Within Lab SD	Within Lab CV%
500	511	8.5	1.7
1000	1041	15.1	1.5

Qualitative imprecision was assessed by taking samples with target values $\pm$ 25 % of the cutoff concentration. The samples were run in duplicates one to two times a day for twenty-two days using one lot number of reagent and one analyzer. The results are in the table below:

Cutoff Value	Sample at $\pm$ 25% Cutoff	Number of observations	Number of correct Results	Confidence Level
500 ng/mL	370 ng/mL	84	84	>95% negative reading
	642 ng/mL	86	86	>95% positive reading
1000 ng/mL	784 ng/mL	86	86	>95% negative reading
	1255 ng/mL	84	84	>95% positive reading

b. Linearity/assay reportable range:

Linearity fluids were prepared from two pools of urine with amphetamine concentrations near the extremes of the calibration range (low pool 0 ng/mL and high pool 1800 ng/mL. The two pools were mixed to create 19 additional pools of intermediate concentrations.

Three determinations of each pool and three determinations of Verifiers were tested with three lots of reagent and one analyzer. A linear regression analysis was performed by the method of least squares. The plotted curve conforms to a straight line, supporting the reportable range 100-1450 ng/mL.

Recovery study:

Fourteen admixtures were prepared from two human urine pools and the concentrations were verified by GC/MS. Results are presented in the table below:

GC/MS ng/mL	VITROS AMPH Assay ng/mL	% Recovery
141	134	95.3
211	216	102.3
281	284	100.8
422	412	97.7
563	566	100.6
704	751	106.8
844	886	104.9
985	1046	106.2
1126	1137	101.0
1196	1213	101.5
1266	1267	100.1
1301	1336	102.6
1337	1349	100.9
1407	1417	100.7

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

A primary calibrator is prepared through gravimetric addition of USP (U.S. Pharmacopoeia) d-methamphetamine reference standard into drug free human urine and the concentration is confirmed by Gas Chromatography/Mass Spectrometry (GC/MS). Next working calibrators are prepared by diluting the primary calibrator with drug free human urine matrix. The value assigned to the working calibrator is verified by GC/MS.

The VITROS DAT Performance Verifiers I,II,III,IV and V are prepared through the gravimetric addition of Sigma catalog number M8750 to drug free human urine matrix and the values are assigned by a GC/MS.

Stability Studies:

Real time and accelerated studies have been conducted. Protocols and acceptance criteria were described and found to be acceptable. The

manufacturer claims the following expiration date:

When stored at 2-8 ºC the assay reagent is good until the expiration date.

When stored at ≤-18 ºC the calibrator is good until the expiration date. Open stored at 2-8 ºC it is good for ≤ 28 days.

When stored at 2-8 ºC the controls are good until the expiration date. Open stored at 2-8 ºC they are good for 4 weeks.

d. Detection limit:

Seven human samples at various concentration below the lowest calibrator (150 ng/mL) were assayed in 10 replicates once a day for 5 days using three different lot numbers of reagent. A fully nested ANOVA was used to determine the total variability of each sample and a pooled SD was calculated for each of the three reagent lots used. The sponsor followed CLSI EP-17A to calculate the limit of quantitation which is 75 ng/mL for this assay.

e. Analytical specificity:

Cross-reactivity was established by spiking various concentrations of similarly structured compounds into drug-free calibrator. The quantity of a compound that produces a value equivalent to the d-methamphetamine (ng/mL) at each cutoff is listed below:

Compound	Quantity equivalent to 500 ng/mL cutoff	% Cross-reactivity	Quantity equivalent to 1000 ng/mL cutoff	% Cross-reactivity
d-methamphetamine	500	100	1,000	100
d-Amphetamine	500	100	1,100	90.9
Benzphetamine	513	97.5	1,032	96.9
l-Methamphetamine	780	64.1	2,300	43.5
l-Amphetamine	3000	16.7	10,000	10
Methylenedioxy-amphetamine	1800	27.8	4,200	23.8
Methylenedioxy-ethylamine	3700	13.5	17,500	5.7
Methylenedioxy-methamphetamine	3400	14.7	20,500	4.9
4-chloramphetamine	4200	11.9	21,000	4.8
Mephentermine	6300	7.9	45,000	2.2

Compound	Quantity equivalent to 500 ng/mL cutoff	% Cross-reactivity	Quantity equivalent to 1000 ng/mL cutoff	% Cross-reactivity
p-hydroxy-amphetamine	10,000	5.0	65,000	1.5
Phentermine	7500	6.7	35,000	2.9
Fenfluramine	32,000	1.6	>100,000	<1.0
Methoxyphenamine	100,000	0.5	>100,000	<1.0
Tranylcypromine	32,000	1.6	>100,000	<1.0
Propanolol	90,000	0.6	>100,000,	<1.0
Tyramine	>100,000	<0.5	>100,000	<1.0
Buproprion	>100,000	<0.5	>100,000	<1.0
l-ephedrine	>100,000	<0.5	>100,000	<1.0
d-ephedrine	>100,000	<0.5	>100,000	<1.0
d-pseudoephedrine	>100,000	<0.5	>100,000	<1.0
l-pseudoephedrine	>100,000	<0.5	>100,000	<1.0
Nor-pseudoephedrine	>100,000	<0.5	>100,000	<1.0
Phenylpropanolamine	>100,000	<0.5	>100,000	<1.0
Chloroquine	>100,000	<0.5	>100,000	<1.0
Phenothiazine	>100,000	<0.5	>100,000	<1.0

To evaluate interference the sponsor spiked potentially interfering compounds into drug-free calibrator. The compounds listed in the table below were found not to interfere according to the sponsor's criterion for bias <95.6 ng/mL at 500 ng/mL and <191 ng/mL at 1000 ng/mL amphetamine:

Compound	Concentration tested ng/mL	Compound	Concentration tested (ng/mL)
Albuterol	100,000	Meperidine	100,000
Ammonia	570	Methylphemidate	100,000
Ascorbic acid	500	Metronidazole	100,000
Bilirubin	26	NaCl	6000
Brompheniramine	100,000	Nylidrine	100,000
Calcium	30	Ofloxacin	100,000
Ciprofloxacin	100,000	Oxalic acid	400
Citric acid	100	pH=4	
Cloxacillin	100,000	pH=9	
Creatinine	300	Phenothiazine	100,000
Dextromethorphan	100,000	Phenyltoloxamine	100,000
Dicyclomine	100,000	Phenylbutazone	100,000

Compound	Concentration tested ng/mL	Compound	Concentration tested (ng/mL)
Diethylproprione	100,000	Phosphate	1420
Desipramine	100,000	Procainamide	100,000
Doxylamine	100,000	Promethazine	100,000
Ethacrynic acid	100,000	Pyruvate	100
Ethanol	780	Quinacrine	100,000
Glucose	4000	Ranitidine	100,000
Hemoglobin	500	Riboflavin	2
Human IgG	200	Setraline	100,000
Human serum albumin	200	Tolmetin/tolectin	100,000
Imipramine	100,000	Trihexylphenidyl	100,000
Indomethacin	100,000	Trimethobenzamide	100,000
Iron	0.1	Tripelannamine	100,000
KCL	1118	Triprolidine	100,000
l-hyoscyamine	100,000	Urea	3000
Magnesium	60	Uric acid	120

Testing of high concentrations of NaCl, albumin and glucose showed no interference for a high specific gravity.

f. Assay cut-off:

Analytical performance of the device around the cutoff is described in Section1.M.d above. The test will yield a positive result when a given drug exceeds this concentration in the urine sample.

2. Comparison studies:

a. Method comparison with predicate device:

One hundred and six unaltered urine samples were assayed using the Vitros Chemistry products AMP reagent. The results were compared with the predicate device and Gas Chromatography/Mass Spectrometry (GC/MS) at the 500 ng/mL cutoff and the 1000 ng/mL cutoff. The results are presented below:

Comparison of Vitros AMP assay to the Predicate

		Commercial Method				% Agreement		
Cutoff Value		Low Negative	Near Cutoff Negative	Near Cutoff Positive	High Positive	% Agreement Negative	% Agreement Positive	% Agreement Overall
500 ng/mL		(<50%) <250 ng/mL	(-50% to cutoff) 250-500 ng/mL	(cutoff to +50%) 500-750 ng/mL	(>+50%) >750 ng/mL	96.3	98.1	97.2
	Vitros Positive	0	2*	5	46			
	Vitros negative	36	16	1*	0			
1000 ng/mL		(<50%) <500 ng/mL	(-50% to cutoff) 500-1000 ng/mL	(cutoff to +50%) 100-1500 ng/mL	(>+50%) >1500 ng/mL	97.4	93.3	96.2
	Vitros Positive	0	2*	7	21			
	Vitros negative	54	20	2*	0			

* See Summary of discordant results below:

Cutoff Value	Vitros AMPH Assay ng/mL	Commercial Method ng/mL
500 ng/mL	491	542
	521	401
	739	477
1000 ng/mL	929	1232
	998	1079
	1021	947
	1061	922

Comparison of Vitros AMP assay to the GC/MS

		Commercial Method				% Agreement		
Cutoff Value		Low Negative	Near Cutoff Negative	Near Cutoff Positive	High Positive	% Agreement Negative	% Agreement Positive	% Agreement Overall
500 ng/mL		(<50%) <250 ng/mL	(-50% to cutoff) 250-500 ng/mL	(cutoff to +50%) 500-750 ng/mL	(>+50%) >750 ng/mL	96.2	96.2	96.2
	Vitros Positive	1*	1*	1	50			
	Vitros negative	42	9	2*	0			
1000 ng/mL		(<50%) <500 ng/mL	(-50% to cutoff) 500-1000 ng/mL	(cutoff to +50%) 100-1500 ng/mL	(>+50%) >1500ng/ mL	98.5	74.4	89.6
	Vitros Positive	0	1*	5	24			
	Vitros negative	53	13	10*	0			

*See Summary of Discordant results below

Cutoff Value	Vitros AMPH Assay ng/mL	GC/MS ng/mL	Major Drug Identified by GC/MS
500 ng/nL	465	555	amphetamine
	491	529	amphetamine
	521	0	none
	739	409	methamphetamine
1000 ng/mL	783	1009	amphetamine
	816	1078	amphetamine
	857	1048	amphetamine
	861	1104	amphetamine
	892	1139	amphetamine
	893	1181	amphetamine
	903	1061	amphetamine
	921	1220	amphetamine
	929	1144	amphetamine
	998	1348	amphetamine
	1303	912	methamphetamine

b. Matrix comparison:

Not Applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not Applicable

*b. Clinical specificity**:***

Not Applicable

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

Not applicable

4. Clinical cut-off**:**

Not Applicable

5. Expected values/Reference range:

Not Applicable

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.