

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

A. 510(k) Number:

k051579

B. Purpose for Submission:

New device

C. Measurand:

Amphetamine

D. Type of Test:

Qualitative immunoassay using oral fluid

E. Applicant:

Immunalysis Corporation

F. Proprietary and Established Names:

Immunalysis Amphetamine ELISA for Oral Fluids

G. Regulatory Information:

1. Regulation section:
21 CFR §862.3100, Amphetamine Test System
2. Classification:
Class II
3. Product code:
DKZ
4. Panel:
Toxicology (91)

H. Intended Use:

1. Intended use(s):
See indications for use below.
2. Indication(s) for use:
The Immunalysis Amphetamine ELISA test system utilizes an Enzyme Linked Immunoassay (ELISA) for the qualitative detection of amphetamine in oral fluid samples collected with the Quantisal™ oral fluid collection device using a cutoff of 50 ng/mL of d-amphetamine. This in vitro diagnostic device is intended for clinical laboratory use only.

The Immunalysis amphetamine ELISA Kit for Oral Fluids provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/ mass spectrometry (GS-MS) is the preferred confirmatory method (1). Clinical and Professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are obtained.

3. Special conditions for use statement(s):
See above.
4. Special instrument requirements:

This device must be read on a spectrophotometer that reads wavelengths of 450 nm and 620 nm.

I. Device Description:

The ELISA assay contains a micro-plate coated with monoclonal mouse antibody to amphetamine (with a spacer chain) and the following reagents: amphetamine conjugate, negative and positive controls, a cut-off calibrator, enzyme-labeled amphetamine, and stop reagent. The device also consists of a saliva collection device. An oral fluid specimen is collected by placing the collection device, a cellulose pad affixed to a propylene stem under the tongue until approximately one milliliter saliva has saturated the pad. A blue indicator on the stem indicates when enough sample has been collected. The collector is transferred to a provided polypropylene tube containing preservative buffer (3 ml) and closed, ready for transport or storage.

J. Substantial Equivalence Information:

1. Predicate device name(s):
DRI Amphetamines EIA Assay
2. Predicate 510(k) number(s):
K934891
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Analyte	Amphetamine	Methamphetamine and Amphetamine
Methodology	Immunoassay (EIA)	Immunoassay (ELISA)

Differences		
Item	Device	Predicate
Test Matrix	Oral Fluid	Urine
Cutoff	50 ng/mL	1000 ng/mL

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

None referenced.

L. Test Principle:

Enzyme-labeled drug and drug present in the sample compete for limited antibody binding sites. Binding of the enzyme-labeled drug inhibits its reaction with the substrate, thereby influencing the rate of absorbance change measured by the instrument. The rate of absorbance change is proportional to the concentration of drug in the sample. Concentrations of controls and unknowns are calculated from the standard curve. Results are read at 450 and 620 nm.

M. Performance Characteristics (if/when applicable):**1. Analytical performance:****a. *Precision/Reproducibility:***

Precision was tested by spiking negative oral fluid with 0, 25, 50, 75, and 100 ng/mL of d-methamphetamine; this corresponded to 0, 50%, 100%, 150%, and 200% of the cutoff. One milliliter of the spiked fluids were pipetted onto the collection pad of the oral fluid collector then processed as per instructions.

Intra-assay precision was estimated with sixteen replicates of each concentration analyzed in one run. Results are shown below:

amphetamine (ng/mL)	Mean OD	Std Dev	CV
0	1.79	0.138	7.71 %
25 (50% c/o)	0.726	0.052	7.29 %
50 (100% c/o)	0.463	0.025	5.45 %
75 (150% c/o)	0.304	0.015	5.18 %
100 (200% c/o)	0.255	0.019	7.49 %

Inter-assay precision was assessed by eight replicates of each concentration run in 10 different assay runs (2 per day over 5 days). Results are expressed as B/B0% where B = absorbance of sample and B0 = absorbance of the zero calibrator.

	amphetamine Concentration (ng/mL)			
	25 (50% c/o)	50 (100% c/o)	75 (150% c/o)	100 (200% c/o)
Mean	34.1	22.2	14.2	12.4
Std.Dev	2.4	1.6	1.1	0.90
% CV	7.1	7.0	8.0	7.2

Reproducibility of the oral fluid collection device was assessed by collecting oral fluid from 50 subjects with a pre-weighed collector and tube as per the package instructions. After the volume indicator turned blue, the collector and tube were weighed and the net weight of the saliva was determined and converted to volume (mLs). Results are shown below:

Avg. Vol. (mL)	Std. Dev.	C.V.	Mean + 3 SD (mL)	Mean - 3 SD (mL)
0.993	0.029	2.88%	1.079	0.907

b. *Linearity/assay reportable range:*

Not applicable. This assay is intended for qualitative use.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
All calibrator and control stock solutions are prepared from commercially available solutions. Amphetamine concentration is confirmed by GC/MS analysis. The error concentration allowed is +/-15% of the nominal concentration. Drug concentrations at 50%, 100% and 200% of the cutoff concentration are stored at 2-8 degrees C for stability testing. Results of testing up to 4 months indicate stability (within +/-15%). Further studies are ongoing.

Stability of specimens within the collection device was determined by spiking a pool of negative saliva with drug at a concentration near the cutoff. Samples were stored at 4°C or at room temperature (22-25°C). The specimens kept at room temperature were assayed in duplicate by GC-MS after 7 days, 14 days and 30 days; samples kept at 4°C were assayed were assayed in duplicate by GC-MS after 14 days and 30 days. Results are shown below:

Day	amphetamine spike (ng/mL)	% initial value	amphetamine spike (ng/mL)	% initial value
0	40.03	100	81.90	100
7	41.30	103.2	80.34	98.1
14	41.57	103.8	72.70	88.77
21	39.04	97.53	76.12	92.94
30	38.40	95.93	69.24	84.54
0	40.03	100	81.90	100
14	40.00	99.9	69.40	84.7
30	36.71	91.7	74.08	90.5

A shipping study showed that recovery of drug in samples after 4 days in transit at temperatures ranging from 69-93°F, was similar to recovery of observed for samples held at 2-8°C.

- d. *Detection limit:*
See the Precision/Reproducibility section above for performance around the stated cutoff concentration.
- e. *Analytical specificity:*
Cross-reactivity of structurally similar compounds was determined by spiking concentrations of potentially cross-reacting drugs into synthetic oral fluid. Results are shown below:

Test compound	Conc. Tested (ng/mL)	Cross-Reactivity
d-amphetamine	25-75	100%
l-amphetamine	250	9.7%
d-methamphetamine	1000	<0.1%
dl-methamphetamine	1000	ND
l-methamphetamine	5000	<0.02%
dl-MDMA	500	ND
dl-MDA	200	179%
dl-MDEA	1000	ND
(+) pseudoephedrine	1000	3.7%
(-) pseudoephedrine	500	<1%
phenolpropanolamine	5000	<0.2
(+) ephedrine	10000	ND
(-) ephedrine	10000	0.42%
fenfluramine	1000	ND
diphenhydramine	10000	ND

Structurally unrelated compounds were spiked into synthetic oral fluid at a concentration of 10000 ng/mL each; none of the compounds tested affected assay results. The list of compounds tested is in the package insert.

Commonly ingested substances were tested for interference. Results are shown below:

Compound	Mean Abs	B/B0%	POS/NEG
Synthetic Oral Fluid	2.799	100	
25 ng/mL amphetamine	1.438	51.38	NEG
50 ng/mL amphetamine	0.735	26.26	Cutoff
100 ng/mL amphetamine	0.499	17.81	POS
	25 ng/mL amp spike		
	Mean Abs	B/B0%	POS/NEG
Distilled water	1.409	50.32	NEG
Sugar water solution	1.268	45.3	NEG
Toothpaste slurry	1.146	41.0	NEG
Cranberry juice	1.164	41.6	NEG
Baking Soda sol'n	1.207	43.1	NEG
Orange juice	0.86	30.8	NEG
Cola	1.015	36.3	NEG
Cough syrup*	1.411	50.4	NEG
Mouthwash	1.217	43.6	NEG

	75 ng/mL amp spike		
	Mean Abs	B/B0%	POS/NEG
Distilled water	0.472	16.9	POS
Sugar water solution	0.439	15.7	POS
Toothpaste slurry	0.610	21.8	POS
Cranberry juice	0.497	17.8	POS
Baking Soda sol'n	0.584	20.9	POS
Orange juice	0.372	13.3	POS
Cola	0.567	20.2	POS
Cough syrup*	0.453	16.2	POS
Mouthwash	0.631	22.6	POS

f. Assay cut-off:

Performance around the assay cut-off of 50 ng/mL is demonstrated in the intra-assay precision section above.

The Substance Abuse and Mental Health Services Administration (SAMHSA) currently recommend 50 ng/mL as a cutoff level for amphetamine oral fluid tests.

2. Comparison studies:

a. Method comparison with predicate device:

Oral fluid and urine samples were collected at the same time from admitted amphetamine users in a clinical setting. Urine samples were tested by the predicate assay using a cutoff of 1000 ng/mL. Oral fluid samples were tested in duplicate using a screening cutoff of 50 ng/mL. All samples were tested by GC/MS at an independent facility. Twenty two samples contained amphetamine (according to GCMS) either below, or near the cutoff concentration.

Comparison of Immunalysis Amphetamine Oral Fluid Assay and the Predicate Urine Assay

		Predicate Urine Assay	
		Pos	Neg
Amphetamine Oral Fluid Assay	Pos	39	2
	Neg	33	111

Comparison of Immunalysis Amphetamine Oral Fluid Assay and GC/MS

		GC/MS	
		Pos	Neg
Amphetamine Oral Fluid Assay	Pos	38	3
	Neg	0	144

Positive agreement: 92.7 %
Negative agreement: 100 %
Overall agreement: 98.3 %

b. Matrix comparison:

Not applicable; this device is intended for use with oral fluid only.

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):
- 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.