

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

A. 510(k) Number:

k051578

B. Purpose for Submission:

New device

C. Measurand:

Cocaine/Cocaine Metabolite

D. Type of Test:

Qualitative immunoassay

E. Applicant:

Immunalysis Corporation

F. Proprietary and Established Names:

Immunalysis Cocaine/Cocaine Metabolite ELISA for Oral Fluids

G. Regulatory Information:

1. Regulation section:

21 CFR §862.3250, Cocaine/Cocaine Metabolite Test System

2. Classification:

Class II

3. Product code:

DIO

4. Panel:

Toxicology (91)

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

“The Immunalysis Cocaine/Cocaine Metabolite ELISA test system utilizes an Enzyme Linked Immunoassay (ELISA) for the qualitative detection of Cocaine/Cocaine Metabolite at a cutoff of 20 ng/mL in ORAL FLUID SAMPLES COLLECTED WITH THE QUANTISAL™ ORAL FLUID COLLECTION DEVICE ONLY. This in vitro diagnostic device is intended for clinical laboratory use only.

The Immunalysis Cocaine/Cocaine Metabolite 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. Clinical and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.”

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 device consists of a saliva collection device and a Cocaine/Cocaine Metabolite ELISA kit. 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. The ELISA assay consists of 8-well microstrips coated with a sheep anti-Benzoyllecgonine and a monoclonal anti-Cocaine antibody, a plate frame, conjugated Cocaine, negative and positive controls, a cut-off calibrator, TMB substrate, and stop reagent.

J. Substantial Equivalence Information:

1. Predicate device name(s):
DRI Cocaine EIA Assay
2. Predicate 510(k) number(s):
K960187
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Analyte	Cocaine and Benzoyllecgonine	Cocaine and Benzoyllecgonine
Methodology	Immunoassay (EIA)	Immunoassay (ELISA)

Differences		
Item	Device	Predicate
Test Matrix	Oral Fluid	Urine
Cutoff	20 ng/mL	300 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 anti-cocaine 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 450nm 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, 10, 20, 30, and 40 ng/mL of Benzoyllecgonine; 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 assessed with sixteen replicates of each concentration analyzed in one run:

Intra-assay Precision: Immunalysis Cocaine/Cocaine Metabolite for Oral Fluid Assay

Benzoyllecgonine concentration (ng/mL)	Mean O.D.	S.D.	C.V.%	B/B0 %
0	2.4351	0.150	6.17	100
10 (50% of cutoff)	1.3943	0.108	7.74	57.26
20(100% of cutoff)	1.2644	0.117	9.28	51.93
30 (150% of cutoff)	1.1456	0.036	3.16	47.04
40 (200% of cutoff)	1.0475	0.059	5.63	43.02

B= Absorbance of the mean concentration

B0=Absorbance of the mean zero dose

Inter-Assay Precision

Negative oral fluid was spiked with 0, 10, 20, 30 and 40 ng/mL of Benzoyllecgonine. This corresponded to 0, 50%, 100%, 150% and 200% of the recommended screening cutoff for Benzoyllecgonine in oral fluids. One mL of these different levels of Benzoyllecgonine spiked oral fluids were pipetted on to the collection pad of the Quantisal Oral Fluid collector and the pad then placed in the transport tube with the preservative buffer.

Eight replicates of the five different Benzoyllecgonine concentrations were analyzed in each run. This was repeated in 10 different assay runs, twice per day over a period of 5 working days.

Benzoyllecgonine	10 ng/mL B/B0 %	20 ng/mL B/B0 %	30 ng/mL B/B0 %	40 ng/mL B/B0 %
MEAN	58.93	48.47	43.18	39.74
S.D.	3.253	3.190	2.98	3.168
C.V. %	5.52	6.58	6.90	7.97

B= Absorbance of the micro-plate well for the particular concentration
 B0= Absorbance of the zero calibrator
 The B/B0 % is a dimensionless number that is independent of the Raw absorbance

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).

Quantisal Oral Fluid Collection Device: Volume Adequacy Study

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

These results support the sponsor’s claim that the device collects 1 mL ± 10% saliva.

- 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 DEA-exempt solutions; Benzoylecgonine concentration is confirmed by GC/MS analysis.

Claimed shelf-life of the oral fluid collection device and the ELISA kit and components is 24 months and 12 months respectively. Real-time studies are ongoing.

Stability of benzoylecgonine in the collection device was determined by spiking a pool of negative saliva with benzoylecgonine at a concentration around the cutoff and a pool at a concentration two-fold higher (2X). Samples were stored at 4°C or at room temperature. 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 in duplicate by GC-MS after 14 days and 30 days. The sponsor’s acceptance criterion was recovery of ± 20% of the initial value.

Stability of Benzoylecgonine in Quantisal Buffer

STABILITY STUDY AT ROOM TEMPERATURE

Data point	BE Spike ng/mL	% of initial value	BE Spike 2X ng/mL	% of initial value
0	13.659	100	30.213	100
7	15.555	113.88	35.089	116.14
14	15.332	112.25	30.102	99.63
21	15.105	110.59	30.271	100.19
30	14.221	104.11	28.785	95.27

STABILITY STUDY AT 4°C

Data point	BE Spike ng/mL	% of initial value	BE Spike 2X ng/mL	% of initial value
0	13.659		30.213	
14	13.078	95.75	33.162	109.76
30	17.367	127.15	28.088	92.97

A shipping study showed that benzoylecgonine spiked into saliva had acceptable recovery ($\pm 15\%$) after transport.

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 different drugs into synthetic oral fluid.

Compound	Concentration ng/mL	B/B0 %	BE equivalents ng/mL	Percent (%) Cross- reactivity
Benzoylecgonine	10	64.6	10	100
Benzoylecgonine	20	47.06	20	100
Benzoylecgonine	40	35.95	40	100
Cocaine	20	50.88	17.26	86.3
Cocaine	40	43.38	25.17	62.92
Cocaethylene	20	48.2	19.62	98.1
Ecgonine	2000	32.54	49.68	2.5
Ecgonine Methyl Ester	2000	93.88	4.05	0.2
Norcocaine	1000	35.11	41.51	4.1

Structurally unrelated compounds were spiked into synthetic oral fluid at a concentration of 10000 ng/mL; none of the compounds in the table below had an immunoassay response greater than the 50% control of the assay (i.e. Benzoyllecgonine at 10 ng/mL).

**Compounds tested for Cross-reactivity:
Immunalysis Cocaine/Cocaine metabolite Oral Fluid Assay**

Acetaminophen	Ethylmorphine	Mereridine
Amitriptyline	Flurazepam	Nalorphine
Amobarbital	Glutethimide	Nicotine
Barbital	Hexobarbital	Nordoxepin
Benzoyllecgonine	Hydromorphone	n-Normethsuximide
Butabarbital	Imipramine	Nortriptyline
Bromazepam	Lidocaine	Oxazepam
Caffeine	Lorazepam	Oxycodone
Carbamazepine	Medazepam	Phenobarbital
Cocaine	Methadone	Phensuximide
Codeine	EDDP	Phenytol
Chlorpromazine	Methaqualone	Primidone
Desipramine	Metharbital	Protriptyline
Diacetylmorphine	Mephentoin	Quinine
Dihydrocarbamazepine	Methyl-propylsuccinimide	Secobarbital
Diazepam	Mephobarbital	Temazepam
Doxepin	Methyl PEMA	Theophylline
Dyphylline	Methsuximide	Trimipramine
Ethosuximide	4-Methylprimidone	
Ethotoin	Morphine	

Commonly ingested substances were tested for interference. The effects of sugar, toothpaste, cranberry juice, baking soda, cola carbonated beverages, cough syrup, mouthwash and distilled water were studied.

Each of these diluted commonly ingested substances was spiked in separate aliquots with 10 ng/mL of Benzoyllecgonine (50% of the cutoff) and 30 ng/mL Benzoyllecgonine (150 % of the cutoff). Quantisal oral fluid collection devices were used with each of the spiked commonly ingested substances and the immunoassay results obtained utilizing the spikes with the above commonly ingested substances were compared to spikes of Benzoyllecgonine in synthetic negative oral fluid.

If a spike at 10 ng/mL of Benzoyllecgonine (50% of the cutoff calibrator) remained negative with respect to the 20 ng/mL Benzoyllecgonine calibrator in synthetic negative oral fluid it was deemed that there was no interference.

If a spike at 30 ng/mL of Benzoyllecgonine (150% of the cutoff calibrator) remained positive with respect to the 20 ng/mL of Benzoyllecgonine calibrator in synthetic negative oral fluid, it was deemed that there was no interference.

Specimen with Benzoylcegonine concentration	Mean Absorbance	B/B0%	POS/NEG	Effect
Zero	2.486	100		
10 ng/mL in Synthetic Oral Fluid	1.709	68.76	NEG	
20 ng/mL in Synthetic Oral Fluid	1.348	54.22	Cutoff	
40 ng/mL in Synthetic Oral Fluid	1.062	42.724	POS	
10ng/mL in sugar water	1.474	59.3	NEG	
10 ng/mL in toothpaste slurry	1.41	56.72	NEG	
10 ng/mL in cranberry juice	1.639	65.96	NEG	
10 ng/mL in baking soda solution	1.842	74.10	NEG	
10 ng/mL in Orange juice	1.539	61.91	NEG	
10 ng/mL in Cola beverage	1.594	64.13	NEG	
10 ng/mL in Cough syrup	1.735	69.79	NEG	
10 ng/mL in mouthwash	1.725	69.39	NEG	
10 ng/mL in Distilled Water	1.743	70.12	NEG	
30 ng/mL in sugar water	1.075	43.23	POS	
30 ng/mL in toothpaste slurry	1.249	50.27	POS	
30 ng/mL in cranberry juice	1.189	47.81	POS	
30 ng/mL in baking soda solution	1.355	54.49	NEG	Negative Effect
30 ng/mL in Orange juice	1.121	45.08	POS	
30 ng/mL in Cola beverage	1.163	46.79	POS	
30 ng/mL in Cough syrup	1.107	44.51	POS	
30 ng/mL in mouthwash	1.185	47.67	POS	
30 ng/mL in Distilled Water	1.153	46.36	POS	

From the above data, only the 30 ng/mL Benzoylcegonine spike in the 25mg/mL baking soda dissolved in Distilled Water had an effect on the immunoassay. Under these conditions, a sample spiked at 150% of the cutoff (i.e. 30 ng/mL of Benzoylcegonine) read as a borderline negative, roughly equivalent to a 20 ng/mL Benzoylcegonine cutoff. None of the other substances tested under these conditions had an effect on the immunoassay.

f. Assay cut-off:

Performance around the assay cut-off of 20 ng/mL is demonstrated in the intra-assay precision section above.

The Substance Abuse and Mental Health Services Administration (SAMHSA) has recommended 20 ng/mL as a cutoff level for cocaine and metabolites oral fluid tests.

2. Comparison studies:

a. Method comparison with predicate device:

Oral fluid and urine samples were collected in the same visit from 100 admitted cocaine users in a clinical setting. Urine samples were tested by the predicate assay using a cutoff of 150 ng/mL. Oral fluid samples were tested in duplicate using a screening cutoff of 20 ng/mL; all samples were tested by GC/MS at an independent facility.

**Comparison of Immunalysis Cocaine/Cocaine metabolite
Oral Fluid Assay and the Predicate Urine Assay**

		Predicate Urine Assay	
		Pos	Neg
Cocaine/Cocaine metabolite Oral Fluid Assay	Pos	53	4
	Neg	4	39

Positive agreement: 93 %
Negative agreement: 91 %
Overall agreement: 92 %

**Comparison of Immunalysis Cocaine/cocaine metabolite
Oral Fluid Assay and GC/MS**

		GC/MS	
		Pos	Neg
Cocaine/Cocaine metabolite Oral Fluid Assay	Pos	50	5
	Neg	2	43

Positive agreement: 91%
Negative agreement: 96 %
Overall agreement: 93 %

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):

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.