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COCAINE METABOLITE INTERCEPT[®] MICRO-PLATE EIA for use with Intercept[®] Oral Fluid Specimens

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INTENDED USE

The OraSure Technologies, Inc. (OTI) Cocaine Metabolite Intercept[®] MICRO-PLATE EIA is intended for use by clinical laboratories in the qualitative determination of cocaine and cocaine metabolites in oral fluid collected with the Intercept[®] Oral Specimen Collection Device. **FOR IN VITRO DIAGNOSTIC USE.**

The OTI Cocaine Metabolite Intercept[®] MICRO-PLATE EIA provides only a preliminary analytical test result. A more specific alternative chemical method should be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry/mass spectrometry (GC/MS/MS) is the preferred confirmatory method.⁽¹⁾ This is a confirmation method that is currently pending SAMHSA acceptance. Clinical consideration and professional judgment should be applied to any drugs of abuse test result, particularly when a preliminary, positive result is observed.

BACKGROUND

Cocaine appears in oral fluid shortly after cocaine use and, depending upon pH and rate of saliva flow, persists for as long as 12-36 hours.^(1, 2) Upon cocaine administration, the major species detected in oral fluid are cocaine, benzoylecgonine, and ecgonine methyl ester.⁽⁴⁾

Cocaine and its metabolites [benzoylecgonine (BE) and ecgonine methyl ester (EME)] can be detected in oral fluid following intravenous administration. Moreover, cocaine enters the salivary glands via the blood circulation and is not present merely as residue following oral or nasal self-administration.⁽²⁾ Quantitative assessment of the excretory pattern of salivary cocaine by GC/MS in controlled-dose studies has revealed that the amount of cocaine in oral fluid consistently exceeds plasma concentrations measured concomitantly.⁽³⁾ In addition, pharmacokinetic studies have shown that cocaine appears in the oral fluid almost immediately following intravenous administration, while BE and EME are only detected at later times and in lower concentrations.^(1, 4) A study conducted by Cone et. al. showed that, after 30 hours following cocaine administration to a single individual, the approximate ratio of cocaine:BE:EME in saliva was 19:1:1.⁽¹⁾ However, additional data regarding the relative quantities of cocaine, BE, and EME in oral fluid is limited. Sufficient studies to provide an accurate determination of the ratios of these compounds in oral fluid have not been conducted.

PRINCIPLE OF THE ASSAY

The OTI Cocaine Metabolite Intercept[®] MICRO-PLATE EIA is a competitive immunoassay for the detection of cocaine and cocaine metabolites in oral fluid collected with the Intercept[®] Oral Specimen Collection Device. Specimen or standard is added to an EIA well in combination with an enzyme-labeled hapten derivative. In an EIA well containing an oral fluid specimen positive for cocaine or cocaine metabolites, there is a competition between cocaine and/or cocaine metabolite and the enzyme labeled hapten to bind the antibody fixed onto the EIA well. EIA wells are then washed, substrate is added, and color is produced. The absorbance measured at 450 nm is inversely proportional to the amount of cocaine or cocaine metabolite present in the specimen or calibrator/control. Because currently there are no SAMHSA assigned cutoffs for cocaine testing using oral fluid, OTI recommends a cutoff of 5.0 ng/mL when testing oral fluid collected with the Intercept[®] Oral Specimen Collection Device. This cutoff is within the limit of detection by the OTI Cocaine Metabolite Intercept[®] MICRO-PLATE EIA.

PRINCIPLE OF THE INTERCEPT[®] ORAL SPECIMEN COLLECTION DEVICE

Saliva is a complex mixture of parotid, submandibular, sublingual and minor salivary gland secretions mixed with mucin, bacteria, leukocytes, sloughed epithelial cells and gingival crevicular fluid.⁽⁵⁾ The fact that cocaine and cocaine metabolites are present in oral fluid following human use has been documented.⁽³⁾

The Intercept[®] Oral Specimen Collection Device was developed for the purpose of collecting oral fluid for diagnostic testing. The collection device consists of a treated absorbent cotton fiber pad affixed to a nylon stick (Collection Pad) and a preservative solution in a plastic container (Specimen Vial). The Collection Pad is impregnated with a mixture of salts and gelatin which creates a hypertonic environment and an increase osmotic pressure wherever it contact oral mucosal cells. The pad is placed in contact with the gingival mucosa (between the lower gum and cheek) which enhances the flow of mucosal transudate across the mucosal surfaces onto the absorptive cotton fibers of the pad. Following the collection period, the Collection Pad is placed into a vial containing a preservative solution which serves to inhibit the growth of oral microorganisms recovered on the Collection Pad. The vial is sealed with a plastic cap and transported to a laboratory for processing and testing. Following processing, a fluid containing a mixture of mucosal transudate, saliva components and the preservative solution is recovered which is suitable for testing for the presence of cocaine an cocaine metabolites in the OTI Cocaine Metabolite Intercept[®] MICRO-PLATE EIA. Refer to the Intercept[®] Oral Specimen Collection Device product insert for specific instructions on the proper collection, handling, and adequacy of oral fluid samples.

KIT COMPONENTS	Catalog No. 1122IB	Catalog No. 1122IC
	480 Test Kit	9600 Test Kit
	Min. Qty.	Min. Qty.
Anti-Cocaine Metabolite Coated Plate – Mouse anti-benzoylcegonine monoclonal antibody immobilized on a polystyrene plate supplied in dry form.	5	100
Enzyme Conjugate - Lyophilized horseradish peroxidase labeled with a benzoylcegonine derivative.	1	1
Conjugate Diluent - Protein matrix of bovine serum with protein stabilizers.	60 mL	750 mL
Substrate Reagent - One bottle containing 3,3', 5,5' tetramethylbenzidine.	60 mL	1 L
Stopping Reagent - Each bottle contains 2 N sulfuric acid.	60 mL	1 L
Oral Fluid Negative Calibrator – Oral Fluid Diluent negative for benzoylcegonine.	2 mL	16 mL
Oral Fluid Negative Control – Oral Fluid Diluent containing 2.5 ng/mL (v/v) benzoylcegonine.	2 mL	16 mL
Oral Fluid Cutoff Calibrator – Oral Fluid Diluent containing 5.0 ng/mL (v/v) benzoylcegonine.	2 mL	16 mL
Oral Fluid Positive Control – Oral Fluid Diluent containing 10 ng/mL (v/v) benzoylcegonine.	2 mL	16 mL

WARNINGS AND PRECAUTIONS

1. The handling of food or drink near the kit reagents is **NOT** recommended.
2. Proper handling of all reagents is strongly advised. It is suggested that disposable materials are used to avoid contamination of Substrate Reagent. Discard Substrate Reagent if obvious blue color develops.
3. Do **NOT** mouth pipet reagents. Handle all specimens and reagents as if potentially infectious.
4. Keep all containers closed when not in use to avoid microbial contamination.
5. Do **NOT** add sodium azide to samples as a preservative.
6. Do **NOT** use reagents past the expiration date.

7. Do **NOT** mix reagents from different kits or manufacturers.
8. Do **NOT** freeze reagents.
9. It is suggested that all OTI reagents be kept out of direct sunlight whenever possible.
10. Stopping Reagent is corrosive; handle with care.

STORAGE/STABILITY OF THE OTI COCAINE METABOLITE INTERCEPT[®] MICRO-PLATE EIA

Store all reagents at 2-8°C until the expiration date indicated on the kit label.

STORAGE/STABILITY OF THE INTERCEPT[®] ORAL SPECIMENS

Oral fluid specimens may be stored at 4°C (39°F) to 37°C (98°F) for a maximum of 21 days. Specimens must be tested in the OTI Cocaine Metabolite Intercept[®] MICRO-PLATE EIA no later than 21 days following specimen collection, assuming that they have been maintained between 4°C and 37°C prior to testing. Specimens may be stored in either the original specimen storage vial or may be maintained as a processed fluid while being stored in a separate storage tube.

INTERCEPT[®] SPECIMEN PROCESSING PROCEDURE

MATERIALS REQUIRED BUT NOT PROVIDED

1. Tubes suitable for centrifuging Intercept[®] Specimen Vials.
2. Centrifuge capable of 600 - 800 x g.

PROCEDURE (Refer to Intercept[®] insert for collection, storage, and shipping instructions)

1. Record the specimen identification number from the Intercept[®] Specimen Vial.
2. Ensure that the specimen is within acceptable dating for testing, i.e. ≤ 21 days from the time of collection.
3. Hold the vial upright with the tip pointed up.
4. Move the pad away from the vial tip by gently tapping the vial.
5. Break the pointed tip of the vial off with your thumb.
6. Place a tube over the vial and invert the tube and vial.
7. Centrifuge at 600 - 800 x g for 15 minutes.
8. Assay or store the resulting eluate according to the procedures described herein.
9. A minimum of 0.7 mL of the eluate sample is required. This can be determined by centrifugation of the samples into graduated containers or by direct pipetting with a calibrated pipet adjusted to the specified volume.
10. If the minimum volume requirement is not met, a new sample should be collected. If this is not possible, a warning should accompany any data generated indicating that an insufficient amount of sample was collected and that this may affect the accuracy of the final result.

ASSAY PROCEDURE

MATERIALS REQUIRED BUT NOT PROVIDED

1. Semi-automated pipets (50 and 100 microliters) with tips.
2. Micro-plate reader capable of reading at a dual wavelength of 450 and 630 nm.
3. Micro-plate washer.
4. Working dilution of Stock Enzyme Conjugate (See "Working Reagent Preparation" instructions below).
5. Intercept[®] eluate sample(s) - 0.7 mL minimum.

WORKING REAGENT PREPARATION

1. Using a calibrated pipet, add 2 mL of Conjugate Diluent to the vial of Lyophilized Stock Enzyme Conjugate.

2. Replace the stopper and gently mix the contents of the vial by inversion for 10 minutes.
3. Using a calibrated pipet, add the volume of reconstituted Stock Enzyme Conjugate specified on the "Lyophilized Stock Enzyme Conjugate Dilution Instructions" for this lot to the Conjugate Diluent bottle.
4. Replace the lid on the bottle and gently mix the contents by inversion for 1 minute. Allow the reagent to equilibrate for 30 minutes at room temperature or overnight at 2-8°C.
5. The working dilution of Stock Enzyme Conjugate is stable for 6 months and may be used in the OTI Cocaine Metabolite Intercept[®] MICRO-PLATE EIA as needed.

PROCEDURE - Note: Allow all reagents and samples to come to room temperature (15-27°C) before use.

1. At the discretion of the operator, all samples, calibrators and controls may be tested in duplicate. The inclusion of calibrators and controls is recommended on each new plate.
2. Add 50 microliters of sample, calibrator, or control to each well. Label wells appropriately.
3. Add 50 microliters of the working dilution of Stock Enzyme Conjugate to each test well.
4. Incubate for 30 minutes at room temperature (15-27°C) in the dark.
5. Wash the plate using a suitable plate washer. As a general rule, wash each well six (6) times with 300 microliters of distilled water.
6. Add 100 microliters of Substrate Reagent to each well and incubate 30 minutes at room temperature (15-27°C) in the dark.
7. Add 100 microliters of Stopping Reagent to each well.
8. Measure the absorbance at 450 nm within 15 minutes of stopping the reaction.

INTERPRETATION

Positive result: Any sample with an absorbance less than or equal to the Oral Fluid Cutoff Calibrator is considered a positive.

Negative result: Any sample with an absorbance greater than the Oral Fluid Cutoff Calibrator is considered a negative.

When interpreting duplicate results, the operator must be aware of several factors which may influence assay results. These include precise pipetting of specimens and reagents, effective washing of plates, and properly calibrated and maintained instrumentation. Duplicate sample results with a difference of 10% or greater between absorbance values should be retested.

QUALITY CONTROL

OTI provides Negative and Positive Controls to monitor the daily performance of the OTI Cocaine Metabolite Intercept[®] MICRO-PLATE EIA. The Oral Fluid Negative Control must have an absorbance greater than the Oral Fluid Cutoff Calibrator, while the Oral Fluid Positive Control must always have an absorbance less than the Oral Fluid Cutoff Calibrator. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

The testing laboratory should also monitor the percent displacement to cutoff between the Oral Fluid Cutoff Calibrator and the Oral Fluid Negative Calibrator (formula listed below). Refer to the Lot Specification Sheet included in each kit for the expected results and acceptable percent displacement criteria. If the kit is not meeting these minimum criteria, contact OTI Technical Service for assistance.

$$\% \text{Displacement to Cutoff} = \frac{A_{450} \text{ Value (Negative Calibrator) } - A_{450} \text{ Value (Cutoff Calibrator)}}{A_{450} \text{ Value (Negative Calibrator)}} \times 100$$

Failure to follow these QC criteria in the OTI Cocaine Metabolite Intercept[®] MICRO-PLATE EIA may cause poor results or otherwise compromise the integrity of the assay.

LIMITATIONS OF THE PROCEDURE

This assay is designed for use with oral fluid collected with the Intercept® Oral Specimen Collection Device. Other samples may produce variable results, and their use is not recommended. In addition, **final pH levels of an oral fluid specimen that are \leq 5.0 may produce false positive results in the assay.** Finally, it is not possible to document all possible effects of oral activities such as eating food, chewing gum, drinking and dental care activities. Therefore, all possible activities that may affect how readily benzoylecgonine is eliminated from saliva to below the cutoff level have not been fully evaluated.

PERFORMANCE CHARACTERISTICS OF ORAL FLUID SPECIMENS

Analytical Sensitivity/Limit Of Detection - The Limit of Detection (LOD) was defined from the signal-to-noise ratio at the zero-drug concentration as the mean zero absorbance (A_0) minus the noise times three ($LOD = A_0 - 3 SD$). The LOD was determined by obtaining the average absorbance value for sixty-four (64) readings of blank Oral Fluid Diluent and calculating the standard deviation (SD) and 3SD of the absorbance. The absorbance value minus 3SD was then extrapolated from the curve and represents the sensitivity of the assay. The LOD was calculated to be 1.5 ng/mL.

Precision - The precision of the OTI Cocaine Metabolite Intercept® MICRO-PLATE EIA was assessed by testing Oral Fluid Diluent containing 0, 2.5, 5.0, and 7.5 ng/mL benzoylecgonine. The intra-assay precision was determined by analyzing each level 16 times per run for 4 runs. Inter-assay precision was determined by analyzing 2 samples at each level twice per day for 20 days. The results of this testing are described in the following table:

BENZOYLECGONINE (ng/mL)	INTRA-ASSAY % CV (n=64)	INTER-ASSAY % CV (n=4/day, 20 days)
0	3.7	8.0
2.5	3.4	9.0
5.0	4.3	9.6
7.5	7.6	10.5

Analytical Specificity/Cross-Reactivity - The following compounds were spiked into Oral Fluid Diluent at a target concentration of 10,000 ng/mL and tested for cross-reactivity. None were found to produce a signal less than or equal to that of the Oral Fluid Cutoff Calibrator.

4-Aminophenyl Sulfone	Cotinine	L-Ephedrine	Pentobarbital
Acetylsalicylic Acid	Cyclizine	L-Methamphetamine	Phencyclidine
Alprazolam	D-Amphetamine	Lidocaine	Phenobarbital
Amobarbital	D-Methamphetamine	Loperamide	Phenylephrine
Ampicillin	Dextromethorphan	Medazepam	Phenylpropanolamine
Atropine	Diacetylmorphine	Meperidine	Procainamide
β -Phenethylamine	Diphenhydramine	Methadone	Procaine
Bupropion	Fenoprofen	Metoprolol	Pseudoephedrine
Butabarbital	Fluoxetine	Morphine	Quinidine
Butalbital	Gemfibrozil	Nalorphine	Salbutamol
Caffeine	Gentisic Acid	Naproxen	Temazepam
Chlordiazepoxide	Glipizide	Niacinamide	Theophylline
Chlorpromazine	Hydrocodone	Norchlordiazepoxide	Tolmetin
Clonazepam	Hydromorphone	Nordiazepam	Δ^9 -THC
Clorazepate	Ibuprofen	Nystatin	Zomepirac
Codeine	Imipramine	Penicillin	

The analytical specificity of an immunoassay is defined as the cross-reactivity of substances in the assay which are structurally related to the target compound. The percent cross-reactivity of a compound in the OTI Cocaine Metabolite Intercept[®] MICRO-PLATE EIA is defined as the apparent benzoylecgonine concentration divided by the spiked concentration times 100.

The cross-reactivity of structurally related compounds was calculated at several spiked concentrations in Oral Fluid Diluent. The following table indicates the apparent concentration of benzoylecgonine for each substance at a concentration which cross-reacted in the assay. Note: Benzoylecgonine was used as the kit standard and, therefore, will exhibit 100% cross-reactivity.

Compound	Tested Concentration (ng/mL)	Benzoylecgonine Equivalents (ng/mL)	Cross Reactivity (%)
Cocaethylene	1	2.0	200
Cocaine	10	6.4	64
Ecgonine	1000	4.4	0.4
Ecgonine Methyl Ester	1000	1.2	0.1
Benzoylecgonine	5	5	100

It is possible that other substances and/or factors not listed above may interfere with the test and cause false results, e.g., technical or procedural errors.

ACCURACY

The relative sensitivity and specificity of the OTI Cocaine Metabolite Intercept[®] MICRO-PLATE assay was determined from 220 oral fluid specimens obtained from clinical laboratories. All samples were tested by EIA and by GC/MS/MS using a 5.0 ng/mL cutoff. The % agreement of the OTI EIA as compared to GC/MS/MS is shown below:

		GC/MS/MS of Intercept [®] Specimens (5.0 ng/mL Cutoff)	
		+	-
OTI Intercept [®] EIA (5.0 ng/mL Cutoff)	+	46	8
	-	7	159

% Agreement = 93.2%

BIBLIOGRAPHY

1. Cone, Edward J., "Saliva Testing for Drugs of Abuse," Addiction Research Center, National Institute on Drug Abuse, Baltimore, Maryland 21224, 1992.
2. Schramm, W., Smith, R.H., and Craig, P.A., "Drugs of Abuse in Saliva: A Review," *Journal of Analytical Toxicology*, 1992; 16:1-9.
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4. Kato, K., Hillgrove, M., Weinhold, L., Gorelick, D.A., Darwin, W.D., and Cone, E.J., "Cocaine and Metabolic Excretion in Saliva Under Stimulated and Nonstimulated Conditions," *Journal of Analytical Toxicology*, 1993; 17:338-341.
5. Intercept[®] Oral Specimen Collection Device, Package Insert. Manufactured by OraSure Technologies, Inc., Beaverton, OR 97008.

Note: Adulteration of reagents, use of instruments without appropriate capabilities, or other failure to follow instructions as set forth in the labeling can affect performance characteristics and stated or implied label claims.