OraSure Technologies, Inc.

150 Webster Street, Bethlehem, PA 18015-1389
Phone: 610-882-1820 ▪ 1-800-869-3538 (USA) ▪ Fax: 610-882-1830
www.orasure.com

AMPHETAMINE-SPECIFIC INTERCEPT™ MICRO-PLATE EIA

for use with Intercept™ Drugs of Abuse (DOA) Oral Fluid Specimens

14001 (01/02)

INTENDED USE

The OraSure Technologies, Inc. (OTI) Amphetamine-Specific Intercept™ MICRO-PLATE EIA is intended for use by clinical laboratories in the qualitative determination of amphetamine in oral fluid collected with the Intercept™ DOA Oral Specimen Collection Device. **FOR *IN VITRO* DIAGNOSTIC USE.**

The OTI Amphetamine-Specific 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 (GC/MS) is the preferred confirmatory method.(1) Clinical consideration and professional judgment should be applied to any drugs of abuse test result, particularly when a preliminary, positive result is observed.

BACKGROUND

Amphetamine has been identified in saliva following administration of d-amphetamine, l-amphetamine or d,l-amphetamine, and may be present in saliva at detectable levels for as long as 50 hours.(2,3,4) Amphetamine enters the salivary glands via the blood circulation and is not present merely as residue following oral self-administration, since good correlation has been found between plasma and saliva drug levels over extended periods.(2) The window of detection for amphetamine in saliva is similar to that in urine although the length of time following drug use for which a positive result may occur in saliva is dependent upon several factors including the frequency and amount of drug, metabolic rate, excretion rate, drug half-life, and the drug user's age, weight, activity, and diet. Generally, detection of amphetamine in urine is successful for 24-48 hours following use, although the time period may extend to several days for chronic users.(3)

PRINCIPLE OF THE ASSAY

The OTI Amphetamine-Specific Intercept™ MICRO-PLATE EIA is a competitive micro-plate immunoassay for the detection of amphetamine in oral fluid collected with the Intercept™ DOA 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 amphetamine, there is a competition between the drug 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 for each well at 450 nm is inversely proportional to the amount of amphetamine present in the specimen or calibrator/control. Because currently there are no SAMHSA assigned cutoffs for amphetamine testing using oral fluid, OTI recommends a cutoff of 100 ng/mL when testing oral fluid collected with the Intercept™ DOA Oral Specimen Collection Device. This cutoff is within the limit of detection by the OTI Amphetamine-Specific Intercept™ MICRO-PLATE EIA.

PRINCIPLE OF THE INTERCEPT™ DOA 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. The fact that amphetamine is present in oral fluid following human use is well documented.(2)

The Intercept™ DOA 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 common salts and gelatin which creates a hypertonic environment and an increased osmotic pressure wherever it contacts 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 micro-organisms 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 saliva components and the preservative solution is recovered which is suitable for testing for the presence of amphetamine in the Amphetamine-Specific Intercept™ MICRO-PLATE EIA manufactured by OraSure Technologies, Bethlehem, PA. Refer to the Intercept™ DOA Oral Specimen Collection Device product insert for specific instructions on the proper collection, handling, and adequacy of oral fluid samples.

REAGENTS PROVIDED

KIT COMPONENTS	Catalog No. 1103IB 480 Test Kit Min. Qty.	Catalog No. 1103IC 9600 Test Kit Min. Qty.
Anti-Amphetamine Coated Plate – Mouse anti-amphetamine monoclonal antibody immobilized on a polystyrene plate supplied in dry form.	5	100
Enzyme Conjugate -- Horseradish peroxidase labeled with an amphetamine hapten diluted in a protein matrix of bovine serum with protein stabilizers.	60 mL	1 L
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 amphetamine.	2 mL	16 mL
Oral Fluid Negative Control – Oral Fluid Diluent containing 50 ng/mL (v/v) d-amphetamine.	2 mL	16 mL
Oral Fluid Cutoff Calibrator – Oral Fluid Diluent containing 100 ng/mL (v/v) d-amphetamine.	2 mL	16 mL
Oral Fluid Positive Control – Oral Fluid Diluent containing 200 ng/mL (v/v) d-amphetamine.	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. Do **NOT** add sodium azide to samples as a preservative.
5. Keep all containers closed when not in use to avoid microbial contamination.
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.

STORAGE/STABILITY OF THE OTI AMPHETAMINE-SPECIFIC INTERCEPT™ MICRO-PLATE EIA

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

STORAGE/STABILITY OF THE INTERCEPT™ DOA 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 Amphetamine-Specific 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™ DOA SPECIMEN PROCESSING PROCEDURE

MATERIALS REQUIRED BUT NOT PROVIDED

1. Tubes suitable for centrifuging Intercept™ DOA Specimen Vials.
2. Centrifuge capable of 600 - 800 x g.

PROCEDURE (Refer to Intercept™ DOA insert for collection, storage, and shipping instructions)

1. Record the specimen identification number from the Intercept™ DOA 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 results.

ASSAY PROCEDURE

MATERIALS REQUIRED BUT NOT PROVIDED

1. Semi-automated pipets (25, 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. Intercept™ DOA eluate sample(s) - 0.7 mL minimum.

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 in each run.
2. Add 25 microliters of sample, calibrator, or control to each well. Label wells appropriately.
3. Add 100 microliters of Enzyme Conjugate to each test well.
4. Incubate for 30 minutes at room temperature *(15-27°C)* in the dark.
5. Using a suitable plate washer, 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 a dual wavelength of 450 and 630 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 provide Negative and Positive Controls to monitor the daily performance of the OTI Amphetamine-Specific 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.

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

LIMITATIONS OF THE PROCEDURE

This assay is designed for use with oral fluid collected with the Intercept™ DOA Oral Specimen Collection Device. Other samples may produce variable results, and their use is not recommended. 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 amphetamine is eliminated from saliva to below the cutoff level have not been fully evaluated.

SPECIFIC PERFORMANCE CHARACTERISTICS OF ORAL FLUID DILUENT

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 - 3SD). The LOD was determined by obtaining the average absorbance value for 80 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 25.5 ng/mL.

Precision - The precision of the OTI Amphetamine-Specific Intercept™ MICRO-PLATE EIA was assessed by testing Oral Fluid Diluent containing 0, 50, 100, 150, and 200 ng/mL amphetamine. 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:

AMPHETAMINE (ng/mL)	MEAN O.D.	INTRA-ASSAY % CV (n=64)	INTER-ASSAY % CV (n=4/day, 20 days)
0	1.905	3.9	6.7
50	1.005	3.5	6.7
100	0.709	4.0	7.5
150	0.563	4.5	7.7
200	0.438	6.4	7.9

Analytical Specificity/Cross-Reactivity -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 Amphetamine-Specific Intercept™ MICRO-PLATE EIA is defined as the apparent amphetamine 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 amphetamine for each substance at a concentration which cross-reacted in the assay. Note: D-Amphetamine was used as the kit standard and, therefore, will exhibit 100% cross-reactivity.

Compound	Tested Concentration (ng/mL)	Amphetamine Equivalents (ng/mL)	Cross-Reactivity (%)
β-Phenethylamine	100	1.15	1.15
Diphenhydramine	1000	5.05	0.51
d-Methamphetamine	1000	9.07	0.91
Doxylamine	10000	0.06	< 0.01
Fenfluramine	10000	4.43	0.04
Isoxsuprine	1000	13.26	1.33
l-Amphetamine	100	48.3	48.3
l-Ephedrine	10000	O/R*	n.d.*
l-Methamphetamine	100	5.33	5.33
l-Phenylalanine	1000	1.25	0.13
PMA	500	161.0	32.2
PMMA	10,000	10.1	0.1
MDA	100	48.93	48.93
MDEA	1000	1.15	0.12
MDMA	10000	0.83	0.01
Mephentermine	100	14.59	14.59
Phentermine	100	4.36	4.36
Phenylephrine	1000	4.29	0.43
Phenylpropanolamine	10000	7.41	0.07
Procaine	10000	O/R*	n.d.*
Pseudoephedrine	100	3.25	3.25

*out of range: not detectable

The user should be aware that the determination of amphetamine equivalents for each compound is only to calculate the % cross-reactivity of these compounds in the assay. For many of these compounds, the absorbance readings obtained were below the limit of detection of 25.5 ng/mL for the assay. As a result, the % cross-reactivities for these compounds at the levels tested are considered estimates only.

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	Cocaethylene	Hydromorphone	Nordiazepam
Acetylsalicylic Acid	Cocaine	Ibuprofen	Nystatin
Alprazolam	Codeine	Imipramine	Penicillin
Amobarbital	Cotinine	Lidocaine	Pentobarbital
Ampicillin	Cyclizine	Loperamide	Phencyclidine
Atropine	Dextromethorphan	Medazepam	Phenobarbital
Benzoylecgonine	Diacetylmorphine	Meperidine	Procainamide
Butabarbital	Diphenhydramine	Methadone	Quinidine
Butalbital	Fenoprofen	Metoprolol	Salbutamol (Albuterol)
Caffeine	Fluoxetine	Morphine	Temazepam
Chlordiazepoxide	Gemfibrozil	Nalorphine	Theophylline
Chlorpromazine	Gentisic Acid	Naproxen	Tolmetin
Clonazepam	Glipizide	Niacinamide	Δ^9-THC
Chlorazepate	Hydrocodone	Norchlordiazepoxide	Zomepirac

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 - Three studies were conducted to determine the clinical accuracy of the OTI Amphetamine-Specific Intercept™ MICRO-PLATE EIA. For oral fluid testing, the cutoffs for EIA and GC/MS were 100 ng/mL and 75 ng/mL, respectively. For urine testing, the cutoffs were 1,000 ng/mL and 500 ng/mL respectively, for the initial screen and GC/MS confirmation based on SAMHSA guidelines.[1]

In the first study, 1568 oral fluid samples were randomly screened. Five (5) specimens were presumed positive by EIA and were tested by GC/MS. Of the 5 presumptive positives, three were positive by GC/MS and contained 95 ng/mL, 115 ng/mL and 381 ng/mL amphetamine. The remaining 2 samples were negative by GC/MS.

In the second study, oral fluid and urine specimen pairs were collected from 229 individuals in a drug rehabilitation clinic. Four (4) specimen pairs were presumed positive by EIA. These samples were confirmed positive by GC/MS and contained 691 ng/mL, 785 ng/mL, 7800 ng/mL and 2500 ng/mL amphetamine.

In the third study, two (2) oral fluid specimens and one urine specimen were collected from 22 individuals who self-reported use of amphetamines in the past 4 days. All oral fluid specimens were tested using the OTI Amphetamine-Specific Intercept™ MICRO-PLATE EIA and confirmed by GC/MS. All urine samples were screened using a commercial immunoassay kit for amphetamines and confirmed using GC/MS. Of the 44 oral fluid samples tested, 14 samples containing 92–798 ng/mL amphetamine were confirmed positive by GC/MS. 26 samples were confirmed negative by GC/MS. 4 samples that were negative by EIA were positive by GC/MS and contained 79 ng/mL, 76 ng/mL, 80 ng/mL and 125 ng/mL amphetamine.

For purposes of calculating % agreement, the data from the three studies were combined as shown below:

		Oral Fluid GC/MS (75 ng/mL) +	Oral Fluid GC/MS (75 ng/mL) -
OTI Oral Fluid EIA (100 ng/mL cutoff)	+	21	2
	-	4	26

% Agreement = 89%

REFERENCES

1. "Urine Testing for Drugs of Abuse," National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
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.
3. Cone, Edward J., "Saliva Testing for Drugs of Abuse," Presented at the NY Acad. Sciences, Oct. 22-25, 1992.
4. Inoue, T. and Seta, S., "Analysis of Drugs in Unconventional Samples," *Forensic Science Review*, 1992; 4(2).
5. Baselt, R.C. and Cravey, R.H., Disposition of Toxic Drugs and Chemicals in Man (Chemical Tox. Inst., CA), 1995.
6. Wan, S.H., et. al., "Kinetics, Salivary Excretion of Amphetamine Isomers, and Effect of Urinary pH," *Clin. Pharmacol. Ther.*, 1978; 23:585-590.

***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.*

***