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BARBITURATES INTERCEPT™ MICRO-PLATE EIA
for use with Intercept™ Drugs of Abuse (DOA) Oral Fluid Specimens

14006 (01/02)

INTENDED USE

The OraSure Technologies, Inc. (OTI) Barbiturates Intercept™ MICRO-PLATE EIA is intended for use by clinical laboratories in the qualitative determination of barbiturates in oral fluid collected with the Intercept™ DOA Oral Specimen Collection Device using a 20 ng/mL cutoff. **FOR IN VITRO DIAGNOSTIC USE.**

> **The OTI Barbiturates 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 drug of abuse test result, particularly when a preliminary, positive result is observed.**

BACKGROUND

Barbiturates are central nervous system (CNS) depressants that are indicated for use as sedatives, hypnotics, anesthetics and anticonvulsants. Barbiturates can be detected in saliva following administration due to a pH-dependent exchange between the blood system and salivary glands. Detection times for barbiturates are dependent upon the specific barbiturate ingested, with saliva detection times being less than that in urine and more closely mimicking the detection times in blood.[2] 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. In urine, detection times can range from 24 hours for the short-acting barbiturates to 3 weeks for the long-acting barbiturates, whereas, in saliva, detection times range from 3–50 hours. [3,4] Barbiturate metabolism depends upon the specific compound, with phenobarbital and barbital undergoing negligible metabolism while others such as secobarbital being extensively metabolized by oxidation. Only 1-5% of the dose is excreted unchanged in the urine.[3]

PRINCIPLE OF THE ASSAY

The OTI Barbiturates Intercept™ MICRO-PLATE EIA is a competitive micro-plate immunoassay for the detection of barbiturates 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 barbiturates, 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 barbiturates present in the specimen or calibrator/control. Because currently there are no SAMHSA assigned cutoffs for barbiturates testing using oral fluid, OTI recommends a cutoff of 20 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 Barbiturates 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 barbiturates are present in oral fluid following human use is well documented.[2,3]

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 barbiturates in the OTI Barbiturates 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. 1108IB**	**Catalog No. 1108IC**
	480 Test Kit	**9600 Test Kit**
	Min. Qty.	**Min. Qty.**
Anti-Barbiturates Coated Plate – Sheep anti-secobarbital polyclonal antibody immobilized on a polystyrene plate.	5	100
Enzyme Conjugate - Horseradish peroxidase labeled with a barbiturate hapten and diluted in a protein matrix with stabilizers.	60 mL	1 L
Substrate Reagent - Contains 3,3', 5,5' tetramethylbenzidine.	60 mL	1 L
Stopping Reagent - Contains 2 N sulfuric acid.	60 mL	1 L
Oral Fluid Negative Calibrator – Oral Fluid Diluent, tested by EIA to be negative for secobarbital.	2 mL	16 mL
Oral Fluid Negative Control – Oral Fluid Diluent containing 10 ng/mL (v/v) secobarbital and tested by EIA.	2 mL	16 mL
Oral Fluid Cutoff Calibrator - Oral Fluid Diluent containing 20 ng/mL (v/v) secobarbital and tested by EIA.	2 mL	16 mL
Oral Fluid Positive Control - Oral Fluid Diluent containing 40 ng/mL (v/v) secobarbital and tested by EIA.	2 mL	16 mL

WARNINGS AND PRECAUTIONS

1. The handling of food or drink near the kit 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 BARBITURATES 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™ 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 Barbiturates 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. It is recommended that users wait at least 10 minutes after ingesting any food, drink or drugs before collecting a sample.
2. Record the specimen identification number from the Intercept™ DOA Specimen Vial.
3. Ensure that the specimen is within acceptable dating for testing, i.e., $\leq$ 21 days from the time of collection.
4. Hold the vial upright with the tip pointed up.
5. Move the pad away from the vial tip by gently tapping the vial.
6. Break the pointed tip of the vial off with your thumb.
7. Place a tube over the vial and invert the tube and vial.
8. Centrifuge at 600 - 800 x g for 15 minutes.
9. Assay or store the resulting eluate according to the procedures described herein.
10. 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.
11. 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. Microplate reader capable of reading at a dual wavelength of 450 and 630 nm.
3. Microplate 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, samples, calibrators / controls can be tested in duplicate. The insertion of calibrators / controls is recommended in each run.
2. Add 50 microliters of sample, calibrator / control to each well. Label wells appropriately.
3. Add 50 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 supplies positive and negative controls to monitor the daily performance of the OTI Barbiturates 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 Barbiturates Intercept™ MICRO-PLATE EIA may cause poor results or otherwise compromise the integrity of the assay.

LIMITATIONS OF 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. A positive result indicates the presence of barbiturate(s) in the sample and does not indicate legal or illegal use. In addition, **final pH levels of an oral fluid specimen that are £ 6.0 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 barbiturates are eliminated from saliva to below the cutoff level have not been fully evaluated.

SPECIFIC EIA PERFORMANCE CHARACTERISTICS OF INTERCEPT™ DOA 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 time three (LOD = A_0 - 3SD). The LOD was determined by obtaining the average absorbance value for 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 8.2 ng / mL.

Precision - The precision of the OTI Barbiturates Intercept™ MICRO-PLATE EIA was assessed by testing the Oral Fluid Diluent containing 0, 10, 20, 30 and 40 ng / mL secobarbital. 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:

Secobarbital (ng/mL)	Mean O.D.	Intra-Assay % CV (n=64)	Inter-Assay % CV (n=4/day, 20 days)
0	1.797	4.1	8.5
10	1.260	4.4	8.9
20	1.072	3.8	8.9
30	0.960	7.1	8.9
40	0.861	4.9	9.4

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 Barbiturates Intercept™ MICRO-PLATE EIA is defined as the apparent secobarbital 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 secobarbital for each substance at a concentration which cross-reacted in the assay.

Compound	Tested Concentration (ng/mL)	Secobarbital Equivalents (ng/mL)	Cross-Reactivity (%)
Allobarbital	100	23.6	23.6
Amobarbital	50	21.6	43.3
Aprobarbital	100	28.9	28.9
Barbital	250	22.7	9.1
Butabarbital	20	36.9	184.7
Butalbital	30	32.8	109.4
Hexabarbital	10,000	6.5	0.1
Mephobarbital	10,000	9.2	0.1
Methohexital	10,000	0.8	0.01
Pentobarbital	50	34.0	68.0
Phenobarbital	60	30.0	50.0
Talbutal	20	34.1	170.4

The following compounds were spiked into Oral Fluid Diluent at a target concentration of 10,000 ng/mL and tested for cross-reactivity. None of the compounds produced a signal less than that of the Oral Fluid Cutoff Calibrator.

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

Interferents - The effect of interfering substances was examined in the OTI Barbiturates Intercept™ MICRO-PLATE EIA. Five (5) subjects consumed/used each adulterant. Oral fluid samples were collected from each volunteer using the Intercept™ DOA Oral Fluid Collection Device after a 5-minute and 10-minute period following consumption/use. Samples were processed and pooled for each interferent and collection time. Aliquots from each sample pool were spiked with 0, 10, 20, 30 or 40 ng/mL secobarbital and tested in the assay. The signals obtained for samples containing only the adulterants were used to assess any effects that may lead to false positive results. The signals of samples containing drug in the presence of each adulterant were used to assess the overall effects of the adulterant. The substance was considered not to interfere if, after the 10 minute waiting period, the samples containing 0 or 10 ng/mL secobarbital produced absorbance readings greater than the 20 ng/mL cutoff and if the samples containing 30 or 40 ng/mL secobarbital produced absorbance readings less than the 20 ng/mL cutoff. Based on these criteria, orange juice and cough syrup were found to produce false negative results in the OTI assay.

Substance	EIA Result @ 10 min. (adulterant only)	EIA Result @ 10 min. (adulterant + secobarbital)
Sugar	No Effect	No Effect
Toothpaste	No Effect	No Effect
Cranberry Juice	No Effect	No Effect
TUMS®	No Effect	No Effect
Orange Juice	No Effect	False Negative
Cola	No Effect	No Effect
Cough Syrup	No Effect	False Negative
Antiseptic	No Effect	No Effect
Water	No Effect	No Effect

The effects of final sample pH or the presence of hemoglobin was also evaluated in the OTI assay. Oral Fluid Diluent containing various levels of secobarbital with a pH range of 3-9 or containing 5 or 10 mg/dL hemoglobin was tested in the EIA. Samples with pH $\leq$ 6 produced false positive results in the assay. Hemoglobin may produce false negative results at the levels tested.

Accuracy – The clinical accuracy of the OTI Barbiturates Intercept™ MICRO-PLATE assay was determined from specimens collected from self-reported barbiturates users and non-users. The cutoff for EIA and GC / MS was 20 ng / mL for oral fluid specimens and 300 ng / mL for urine specimens.

A total of 128 oral fluid and urine specimen pairs were collected. All oral fluid and urine samples were tested by EIA. Of the 128 samples collected, 127 oral fluid samples were confirmed by GC / MS. All urine samples that were positive by EIA and approximately 10% of the EIA negatives were confirmed by GC / MS. The oral fluid and urine results compared as follows:

		GC / MS of Intercept™ Specimens (20 ng / mL cutoff)	
		+	-
OTI Intercept™ EIA (20 ng / mL cutoff)	+	57	1
	-	2	67

% Agreement = 97.6%

		Urine EIA (300 ng / mL cutoff)	
		+	-
OTI Intercept™ EIA (20 ng / mL cutoff)	+	51	8
	-	2	67

% Agreement = 92.2%

Near cutoff validation – A total of 57 negative Intercept™ oral fluid samples were spiked with various levels of secobarbital ranging from approximately 50% above to 50% below the cutoff concentration (as determined by GC / MS). All samples were screened by EIA and confirmed by GC / MS. Of the 57 samples tested, 44 samples (77%) had EIA results that agreed with the GC / MS values. Eight of the remaining 13 samples gave positive results by EIA but were negative by GC / MS and 5 gave negative results by EIA but were positive by GC / MS.

BIBLIOGRAPHY

1. "Urine Testing for Drugs of Abuse," National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
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3. Schramm, W., Smith, R.H., and Craig, P.A., "Drugs of Abuse in Saliva: A Review," *Journal of Analytical Toxicology*, 1992; 16:1-9.
4. Cone, E.J., "Saliva Testing for Drugs of Abuse," Presented at the NY Acad. Sciences meeting Oct. 22-25, 1992.
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.*