OraSure Technologies, Inc.

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

CANNABINOIDS INTERCEPT ä MICRO-PLATE EIA

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

14021 (03/02)

INTENDED USE

The OraSure Technologies, Inc. (OTI) Cannabinoids Intercept™ MICRO-PLATE EIA is intended for use by clinical laboratories in the qualitative determination of cannabinoids in oral fluid collected with the Intercept™ DOA Oral Specimen Collection Device using a 1.0 ng/mL cutoff. **FOR IN VITRO DIAGNOSTIC USE.**

The OTI Cannabinoids Intercept ä 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. 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

THC and metabolites appear in saliva shortly after use and, depending upon pH and rate of saliva flow, persist in saliva for as long as 14 hours[1] and may be used as an indication of current intoxication.[2] In contrast, urine cannabinoid assays are useful only for the identification of cannabinoid use sometime in the past, with the urine detection period being highly variable and depending on time, dose, body weight, obesity, and habit.[2] After smoking marijuana, saliva may contain THC-COOH and three metabolites; Δ^9-THC, cannabidiol, and 11-hydroxy-Δ^9-THC.[1] THC and its metabolites appear to be sequestered in the buccal cavity during smoking, rather than passing from the blood into saliva.[3] 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. The OTI Cannabinoids Intercept™ MICRO-PLATE EIA detects a variety of cannabinoids found in oral fluids.

PRINCIPLE OF THE ASSAY

The OTI Cannabinoids Intercept™ MICRO-PLATE EIA is a competitive micro-plate immunoassay for the detection of cannabinoids 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 cannabinoids, 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 cannabinoids present in the specimen or calibrator/control. Because currently there are no SAMHSA assigned cutoffs for cannabinoids testing using oral fluid, OTI recommends a cutoff of 1.0 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 Cannabinoids 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.[4] The fact that cannabinoids are present in oral fluid following human use is well documented.[2]

The Intercept™ DOA Oral Fluid 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. When used properly, the Oral Specimen Collection Device will collect an average of 0.4 mL (range ± 0.05 – 0.7 mL, n = 83) of oral fluid. 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 cannabinoids in the OTI Cannabinoids 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.

KIT COMPONENTS	Catalog No. 1118IB 480 Test Kit Min. Qty.	Catalog No. 1118IC 9600 Test Kit Min. Qty.
Anti-THC Coated Plate – Sheep anti-cannabinoids polyclonal antibody immobilized on a polystyrene plate supplied in dry form.	5	100
THC Enzyme Conjugate – Horseradish peroxidase labeled with a Δ^9-THC derivative diluted in a protein matrix with stabilizers.	30 mL	560 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
THC Pre-Buffer -- 50% methanol solution	40 mL	750 mL
Oral Fluid Negative Calibrator – Oral Fluid Diluent tested by EIA to be negative for Cannabinoids.	2 mL	16 mL
Oral Fluid Negative Control – Oral Fluid Diluent containing 0.5 ng/mL (v/v) Δ^9 THC and tested by EIA.	2 mL	16 mL
Oral Fluid Cutoff Calibrator – Oral Fluid Diluent containing 1.0 ng/mL (v/v) Δ^9 THC and tested by EIA.	2 mL	16 mL
Oral Fluid Positive Control -- Oral Fluid Diluent containing 2.0 ng/mL (v/v) Δ^9 THC and tested by EIA.	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 reagents be kept out of direct sunlight whenever possible.

STORAGE/STABILITY OF THE OTI CANNABINOIDS 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 Cannabinoids 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., ≤ 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 (25, 50 and 100 microliters) with tips.
2. Micro-plate reader capable of reading at 450 nm 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 25 microliters THC Pre-Buffer to each well.
4. Incubate for 60 minutes at room temperature (*15-27°C*).
5. Add 50 microliters of Enzyme Conjugate to each test well.
6. Incubate for 30 minutes at room temperature *(15-27°C)* in the dark.
7. Using a suitable plate washer, wash each well six (6) times with 300 microliters of distilled water.
8. Add 100 microliters of Substrate Reagent to each well and incubate 30 minutes at room temperature *(15-27°C)* in the dark.
9. Add 100 microliters of Stopping Reagent to each well.
10. Measure the absorbance at 450 nm 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. It is recommended that duplicate sample results with a difference of 10% or greater between absorbance values be retested.

QUALITY CONTROL

OTI provides Negative and Positive Controls to monitor the daily performance of the OTI Cannabinoids 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.

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

Failure to follow these QC criteria in the OTI Cannabinoids 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™ DOA 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 £ 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 the THC is eliminated from saliva to below the cutoff level have not been fully evaluated.

SPECIFIC EIA PERFORMANCE CHARACTERISTICS

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 0.37 ng / mL.

Precision – The precision of the OTI THC Intercept™ MICRO-PLATE EIA was assessed by testing the Oral Fluid Diluent containing 0, 0.5, 1.0, 1.5 and 2.0 ng / mL Δ^9-THC. Intra-assay precision was determined by analyzing each level 16 times per run for 4 runs. Inter-assay precision was determined by analyzing 2 replicates for each level twice per day for 20 days. The results of this testing are described in the following table:

Δ^9-THC (ng / mL)	Mean O.D.	Intra-Assay %CV (n=64)	Inter-Assay % CV (n=4/day, 20 days)
0	2.105	4.7	8.7
0.5	1.657	4.5	9.3
1.0	1.269	5.2	11.0
1.5	0.997	5.5	11.6
2.0	0.837	4.6	10.8

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

Compound	Tested Concentration (ng / mL)	Δ^9-THC Equivalents (ng / mL)	Cross-Reactivity (%)
Cannabidiol	500	1.36	0.3
Cannabinol	10	1.51	15.1
Δ^8-THC	1	1.06	105.5
11-Hydroxy-Δ^9-THC	1	1.74	174.1
11-nor-9-carboxy-Δ^9 THC	0.5	1.40	279.4

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

4-Aminophenyl Sulfone
Acetylsalicylic Acid
Alprazolam
Amobarbital
Ampicillin
Atropine
β-Phenethylamine
Benzoylecgonine
Butabarbital
Butalbital
Caffeine
Chlordiazepoxide
Chlorpromazine
Clonazepam
Clorazepate
Cocaethylene
Cocaine
Codeine
Cotinine
Cyclizine
D-Amphetamine
D-Methamphetamine
Dextromethorphan
Diacetylmorphine
Diphenhydramine
Fenoprofen
Fluoxetine
Gemfibrozil
Gentisic Acid
Glipizide
Hydrocodone
Hydromorphone
Ibuprofen
Imipramine
L-Ephedrine
L-Methamphetamine
Lidocaine
Loperamide
Medazepam
Meperidine
Methadone
Metoprolol
Morphine
Nalorphine
Naproxen
Niacinamide
Norchlordiazepoxide
Nystatin
Penicillin
Pentobarbital
Phencyclidine
Phenobarbital
Phenylephrine
Phenylpropanolamine
Procainamide
Procaine
Pseudoephedrine
Quinidine
Salbutamol (Albuterol)
Temazepam
Theophylline
Tolmetin
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 (sugar, toothpaste, cranberry juice, orange juice, antacid, cola, cough syrup, antiseptic and water) was examined in the OTI Cannabinoids Intercept™ MICRO-PLATE EIA. For each adulterant, Intercept™ DOA Oral Fluid Collection Devices were spiked with either the adulterant only or the adulterant and THC (corresponding to 0.5, 1.0 or 1.5 ng/mL of THC after collection of the eluate), processed according to the product instructions 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. None of the materials interfered in the assay at the levels tested.

The effects of final sample pH or the presence of hemoglobin was also evaluated in the assay. Oral Fluid Diluent containing 0, 0.5, 1.0, or 1.5 ng/mL THC 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.

Accuracy – The clinical accuracy of the OTI Cannabinoids Intercept™ MICRO-PLATE assay was determined from specimens collected from known drug users. The cutoffs for EIA and GC/MS/MS were 1.0 ng/mL and 0.5 ng/mL, respectively, for oral fluid specimens. The cutoffs for EIA and GC/MS were 50 ng/mL and 15 ng/mL, respectively, for urine specimens.

105 oral fluid and urine sample pairs were collected from 12 subjects (9 marijuana smokers, 3 non-users). Sample collection occurred over a 3-day period following drug administration. An additional 99 oral fluid and urine sample pairs were collected from self-admitting marijuana users (route of administration unknown) or suspected users. All oral fluid and urine samples were analyzed by EIA. Oral fluid samples were confirmed by GC/MS/MS. All EIA-positive urine samples and approximately 50% of EIA-negative urine samples were confirmed by GC/MS. The oral fluid and urine results compared as follows:

		GC/MS/MS of Intercept™ Specimens (0.5 ng/mL cutoff)	
		+	-
OTI Intercept™ EIA (1.0 ng/mL cutoff)	+	82	10
	-	2	110

% Agreement = 94.1%

		Urine EIA (50 ng/mL cutoff)	
		+	-
OTI Intercept™ EIA (1.0 ng/mL cutoff)	+	48	44
	-	37	75

% Agreement = 60.3%

The low agreement between the oral fluid and urine screen results can be attributed to differences in drug deposition into the two matrices and the administrative cutoff in urine.

Near cutoff validation – A total of 58 negative Intercept™ oral fluid samples were spiked with various levels of THC ranging from approximately 80% above and 90% below the cutoff concentration (as determined by GC/MS/MS). All samples were screened by EIA and confirmed by GC/MS/MS. The results compared as follows:

		GC/MS/MS (0.5 ng/mL cutoff) +	GC/MS/MS (0.5 ng/mL cutoff) -
OTI Intercept™ EIA (1.0 ng/mL cutoff)	+	14	0
	-	30	14

% Agreement = 48%

REFERENCES

1. Schramm, W., Smith, R.H., and Craig, P.A., "Drugs of Abuse in Saliva: A Review," *Journal of Analytical Toxicology 1992;* 16:1-9.
2. Samyn, N., et al., "Analysis of Drugs of Abuse in Saliva," *Forensic Science Review*, 11(1): 1999.
3. Inoue, T. and Seta, S., "Analysis of Drugs in Unconventional Samples," Forensic Science Review, Vol. 4, No. 2, Dec. 1992.
4. Intercept™ Drugs of Abuse Oral Specimen Collection Device, Package Insert. Manufactured by OraSure Technologies, Inc., Beaverton, OR 97008.
5. Cone, Edward, J., "Saliva testing for drugs of abuse," Addiction Research Center, National Institute on Drug Abuse, Baltimore, Maryland 21224, 1992.

***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.*

***