CEDIA® Amphetamine OFT Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use Only**
(For Use with Oral-Eze® Oral Fluid Collection System)

Rx Only

REF 10018579 (3 x 17 mL Kit)
10014947 (65 mL Kit)
10021729 (495 mL Kit)

Intended Use
The Thermo Scientific CEDIA Amphetamine OFT Assay is intended for use in the qualitative determination of amphetamine in human oral fluid at a cutoff concentration of 150 ng/mL in neat oral fluid. The specimen must be collected exclusively with the Oral-Eze® Oral Fluid Collection System. The assay is calibrated against d-amphetamine and performed on clinical chemistry analyzers. This in vitro diagnostic device is intended for clinical laboratory use only.

The CEDIA® Amphetamine OFT Assay provides only a preliminary analytical test result. A more specific alternative method must be used to obtain a confirmed analytical result. Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) are the preferred confirmatory methods.[1-3] Clinical consideration and professional judgment should be applied to any drug of abuse test result particularly when preliminary positive results are used.

Summary and Explanation of the Test
The collection of oral fluid is less invasive and no special facilities are required. Oral fluid contains mostly parent drug and therefore is a better indicator of recent drug use.

Amphetamine is generally self-administered either by nasal inhalation or nasal ingestion. The concentration of amphetamine is about three times higher in saliva than in plasma and can be detected in saliva up to 50 hours.[4] Because of the high concentration of amphetamine in saliva and the effect of urine pH on the excretion of drug, saliva is a better medium for the determination of amphetamine.[5]

CEDIA Amphetamine OFT Assay uses recombinant DNA technology to produce a unique homogeneous enzyme immunoassay system.[6] The assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. These fragments spontaneously re-associate to form fully active enzyme that, in the assay format, cleave a substrate, generating a color change that can be measured spectrophotometrically.

In the assay, analyte in the sample competes with analyte conjugated to one inactive fragment (enzyme donor) of β-galactosidase for antibody binding site. If analyte is present in the sample, it binds to antibody, leaving the inactive enzyme fragment free to form active enzyme. If the analyte is not present in the sample, antibody binds to analyte conjugated on the inactive fragment, inhibiting the re-association of inactive β-galactosidase fragments, and no active enzyme is formed. The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of analyte present in the sample.

Reagents
1. **EA Reconstitution Buffer**
   Contains buffer salts, 1.5 mg/L mouse monoclonal anti-amphetamine antibody, stabilizer and preservative.

1a **EA Reagent**
   Contains 0.171 g/L Enzyme Acceptor (microbial), buffer salts and preservative.

2. **ED Reconstitution Buffer**
   Contains buffer salts, stabilizers, and preservative.

2a **ED Reagent**
   Contains 0.135 mg/L Enzyme Donor (microbial) conjugated to amphetamine derivative, 1.67 g/L chlorophenol red-β-D-galactopyranoside, stabilizers, detergent and preservative.

Additional Materials Required (sold separately)

REF	*Kit Description*
10014954	CEDIA Multi-Drug OFT Negative Calibrator
10014955	CEDIA Multi-Drug OFT Cutoff Calibrator
10014957	CEDIA Multi-Drug OFT Controls Kit
96100-050	Oral-Eze Collection Device (50/Box)
96100-500	Oral-Eze Collection Device (500/Box)
96105-050	Oral-Eze Sample Extractor (50/Box)
96105-500	Oral-Eze Sample Extractor (500/Box)

⚠ Precautions and Warnings
This test is for in vitro diagnostic use only. The reagents are harmful if swallowed.

DANGER: Powder reagent contains ≤56% w/w Bovine serum albumin (BSA) and ≤2% w/w Sodium azide. Liquid reagent contains ≤1.0% Bovine serum, ≤0.3% Sodium azide and ≤0.1% Drug-specific antibody (Mouse).

H317 - May cause allergic skin reaction.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.
EUH032 - Contact with acids liberates very toxic gas.

Avoid breathing dust/mist/vapors/spray. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/ eye protection/face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. If skin irritation or rash occurs: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/ container to location in accordance with local/regional/national/international regulations.

Do not use the reagents beyond their expiration dates.

Reagent Preparation and Storage
For preparation of the solutions, refer to the section below. Remove the kit from refrigerated storage (2-8°C) immediately prior to preparation of the solutions.

In the case of accidental spill, clean and dispose of material according to your laboratory's SOP, local, state, and country regulations, with consideration that the material contains potentially infectious materials.

In the case of damaged packaging on arrival, contact your technical support representative (refer to back page of this PI).

Prepare the solutions in the following order to minimize possible contamination.

R2 Enzyme donor solution
Connect Bottle 2a (ED reagent) to Bottle 2 (ED Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 2a is transferred into Bottle 2. Avoid the formation of foam. Detach Bottle 2a and adapter from Bottle 2 and discard. Cap Bottle 2 and let stand approximately 5 minutes at room temperature (21-25°C). Mix again. Record the reconstitution date on the bottle label. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage (2-8°C) and let stand 30 minutes before use.

R1 Enzyme acceptor solution
Connect Bottle 1a (EA reagent) to Bottle 1 (EA Reconstitution Buffer) using one of the enclosed adapters. Mix by gentle inversion, ensuring that all the lyophilized material from Bottle 1a is transferred into Bottle 1. Avoid the formation of foam. Detach Bottle 1a and adapter from Bottle 1 and discard. Cap Bottle 1 and let stand approximately 5 minutes at room temperature (21-25°C). Mix again. Record the reconstitution date on the bottle label. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage (2-8°C) and let stand 30 minutes before use.

NOTE 1: The components supplied in this kit are intended for use as an integral unit. Do not mix components from different lots.

NOTE 2: Avoid cross-contamination of reagents by matching reagent caps to the proper reagent bottle. The R2 solution (Enzyme Donor) should be yellow-orange in color. A red or red-purple color indicates that the reagent has been contaminated and must be discarded.

NOTE 3: The R1 and R2 solutions must be at the reagent compartment storage temperature of the analyzer before performing the assay. Refer to the analyzer specific application sheet for additional information.

Store reagents at 2-8°C. **DO NOT FREEZE.** For stability of the unopened components, refer to the box or bottle labels for the expiration date.

R1 Solution: 60 days refrigerated on analyzer or at 2-8°C.
R2 Solution: 60 days refrigerated on analyzer or at 2-8°C.

Specimen Collection and Handling
Oral fluid samples are suitable for use in the CEDIA Amphetamine OFT Assay. Collect oral fluid samples using the Oral-Eze Oral Fluid Collection System. Care should be taken to preserve the chemical integrity of the oral fluid sample from the time it is collected until the time it is assayed by securely capping the samples, storing the samples at 2-8°C or at room temperature (21-25°C), and testing within 21 days after collection.

Handle oral fluid samples as if they were potentially infectious.
Samples within a pH range of 5-9 are suitable for testing with this assay.

Oral-Eze Sample Processing Procedure
1. Label the sample collection vial with proper identification.
2. Check the sample collection date on the vial to ensure that the sample is within 21 days from the date of collection.
3. Open the cap and compress the pad to express the sample.
4. Recap the vial and the sample is ready for testing.
5. Ensure that the oral fluid samples are maintained between 4°C and 37°C during shipping.
6. Samples can be stored at room temperature (21-25°C) for 21 days. They should be stored at 2-8°C.

Assay Procedure
The Oral-Eze Oral Fluid Collection Device contains a preservative buffer that dilutes the neat oral fluid sample. The calibrator and control levels are set at diluted levels so that sample absorbance values can be compared directly to the absorbance value of the cutoff calibrator. The assay result is reported as a positive or negative result relative to the neat oral fluid cutoff of 150 ng/mL. The concentrations reported in this insert refer to the neat oral fluid concentration, unless otherwise noted.

NOTE: To correlate the Oral-Eze result from the assay or the associated LC-MS/MS confirmation result to a neat oral fluid value, the result from the Oral-Eze sample should be multiplied by a factor of 3.

1. Pipet the processed oral fluid samples and controls into labeled sample cups and place the cups into the sample ring of the MGC 240 analyzer.
2. Load reagents (reagent 1 and reagent 2) into the reagent compartment of the analyzer.
3. Pipet calibrators into labeled cups and load the cups into the sample ring of the analyzer.
4. Program the run setup using 570 nm as primary wavelength and 660 nm as the secondary wavelength. Refer to the parameter sheet for detailed instructions on how to program the analyzer.
5. Use the cutoff calibrator as a reference for distinguishing negative from positive samples.

Quality Control

The CEDIA Multi-Drug OFT Controls are designed to be used with the CEDIA Amphetamine OFT Assay. Good laboratory practice suggests the use of control specimens to ensure proper assay performance. Ensure that controls results are within the established ranges determined by laboratory practices and guidelines. If control results fall outside of established ranges, assay results are invalid. All quality control requirements should be performed in conformance with local, state, and/or federal regulations or accreditation requirements.

Interpretation of results

A sample that exhibits a change in absorbance (ΔA) value equal to or greater than the value obtained with the cutoff calibrator is considered positive. A sample that exhibits a change in absorbance (ΔA) value lower than the value obtained with the cutoff calibrator is considered negative.

Extraction Efficiency and Drug Recovery

Extraction efficiency of the preservative buffer in recovering the drug from the collection device pad was determined by testing the recovery of the drug by LC-MS/MS method. The oral fluid samples at the cutoff level and ±50% control levels recovered within ±20% from nominal values.

Limitations

A positive result from this assay indicates only the presence of amphetamine and does not necessarily correlate with the extent of physiological and psychological effects.

It is possible that other substances and/or factors (e.g.: technical or procedural), other than those investigated in the specificity study may interfere with the test and cause false results.

Specific Performance Characteristics

Typical performance results obtained on the MGC 240 analyzer are shown below. The results obtained in your laboratory may differ from these data.

Precision and Cutoff Characterization

Negative neat oral fluid samples were collected and then prepared by spiking amphetamine at negative, -75%, -50%, -25%, below the cutoff, at the cutoff, and +25%, +50%, +75% and +100% above the cutoff. All spiked neat oral fluid sample concentrations were confirmed by LC-MS/MS. The neat oral fluid samples were processed using the Oral-Eze® device to obtain diluted oral fluid samples. The diluted oral fluid samples were confirmed by LC-MS/MS and tested in the CEDIA® Amphetamine OFT Assay in qualitative mode.

The randomized CLSI (EP5-A2) precision protocol was followed with five replicates of each sample for each run, 2 runs per day for five non-consecutive days, total N= 50/level.

The results are summarized in the table below.

Analyte	Tested Concentration (ng/mL)	Neat Oral Fluid LC-MS/MS (ng/mL)	Diluted Oral Fluid LC-MS/MS (ng/mL)	Amphetamine OFT Assay # Neg / # Pos
Amphetamine	0	< LOQ	< LOQ	50 Neg / 0 Pos
Amphetamine	37.5	43.79	14.75	50 Neg / 0 Pos
Amphetamine	75	88.18	26.84	50 Neg / 0 Pos
Amphetamine	112.5	131.69	40.31	50 Neg / 0 Pos
Amphetamine	150	177.67	51.76	6 Neg / 44 Pos
Amphetamine	187.5	214.37	62.8	0 Neg / 50 Pos
Amphetamine	225	260.91	75.91	0 Neg / 50 Pos
Amphetamine	262.5	305.93	91.82	0 Neg / 50 Pos
Amphetamine	300	296.86	107.47	0 Neg / 50 Pos

Specificity and Cross-Reactivity

Compounds used in over-the-counter cold medicines and other compounds that are structurally related to amphetamine were tested for cross-reactivity in the assay. The cross-reactant solutions were prepared by adding the compounds to neat oral fluid samples at the concentration listed in the table below. The neat oral fluid samples were processed using the Oral-Eze device to obtain diluted oral fluid samples which were tested in the CEDIA Amphetamine OFT Assay. The concentrations listed below were the highest levels yielding negative results in the assay.

Compounds	Tested Concentration in Neat Oral Fluid (ng/mL)	Amphetamine OFT Assay Negative / Positive
d,l -amphetamine	240	Negative
l -amphetamine	6,000	Negative
Phenethylamine	1,950	Negative
Diphenhydramine	3,000,000	Negative

(Con't)

Compounds	Tested Concentration in Neat Oral Fluid (ng/mL)	Amphetamine OFT Assay Negative / Positive
Doxylamine	3,000,000	Negative
d -Ephedrine	3,000,000	Negative
l -Ephedrine	3,000,000	Negative
d,l -Ephedrine	3,000,000	Negative
Fenfluramine	300,000	Negative
Isoxsuprine	3,000,000	Negative
d -Methamphetamine	225,000	Negative
l -Methamphetamine	900,000	Negative
d,l -Methamphetamine	360,000	Negative
PMA	30,000	Negative
PMMA	30,000	Negative
MDA	90,000	Negative
MDEA (3,4-MDE)	600,000	Negative
MDMA	600,000	Negative
Mephentermine	600,000	Negative
Phentermine	9,000	Negative
Phenylephrine	3,000,000	Negative
Phenylpropanolamine	90,000	Negative
Procaine	3,000,000	Negative
d-Pseudoephedrine	3,000,000	Negative
l-Pseudoephedrine	3,000,000	Negative

Various common over-the-counter medications and structurally unrelated compounds were tested for cross-reactivity in the assay. The cross-reactant solutions were prepared by adding the compounds to neat oral fluid samples at the concentration listed in the table below. The neat oral fluid samples were processed using the Oral-Eze device to obtain diluted oral fluid samples which were tested in the CEDIA Amphetamine OFT Assay. All the compounds tested negative and did not show any cross reactivity.

Compounds	Tested Concentration in Neat Oral Fluid (ng/mL)	Amphetamine OFT Assay Negative / Positive
Acetaminophen	60,000	Negative
Acetylsalicylic Acid	60,000	Negative
Alprazolam	30,000	Negative
Amobarbital	30,000	Negative
Amoxicillin	12,000	Negative
Ampicillin	30,000	Negative
Atropine	30,000	Negative
Benzoylecgonine	60,000	Negative
Butabarbital	30,000	Negative
Butabital	30,000	Negative
Caffeine	60,000	Negative
Captopril	60,000	Negative
Chlordiazepoxide	60,000	Negative
Chlorpromazine	30,000	Negative
Cimetidine	60,000	Negative
Clonazepam	30,000	Negative
Clorazepate	30,000	Negative
Cocaine	30,000	Negative
Codeine	12,000	Negative
l -Cotinine	30,000	Negative
Cyclizine	30,000	Negative
Dextromethorphan	30,000	Negative
Diazepam	60,000	Negative
Digoxin	12,000	Negative

(Con't)

Compounds	Tested Concentration in Neat Oral Fluid (ng/mL)	Amphetamine OFT Assay Negative / Positive
Enalapril	60,000	Negative
Fluoxetine	60,000	Negative
Gentisic Acid	30,000	Negative
Hydrocodone	30,000	Negative
Hydromorphone	30,000	Negative
Ibuprofen	60,000	Negative
Imipramine	30,000	Negative
Levothyroxine	6,000	Negative
Lidocaine	30,000	Negative
Loperamide	30,000	Negative
Medazepam	30,000	Negative
Meperidine	30,000	Negative
Methadone	60,000	Negative
Metoprolol	30,000	Negative
Morphine	12,000	Negative
Nicotine	30,000	Negative
Nifedipine	60,000	Negative
Norchlordiazepoxide	30,000	Negative
Nordiazepam	30,000	Negative
Penicillin	30,000	Negative
Pentobarbital	30,000	Negative
Phencyclidine	60,000	Negative
Phenobarbital	60,000	Negative
Procainamide	30,000	Negative
Propoxyphene	60,000	Negative
Ranitidine	12,000	Negative
Salicyluric Acid	60,000	Negative
Secobarbital	60,000	Negative
Temazepam	30,000	Negative
Theophylline	30,000	Negative
Tolmetin	30,000	Negative
Δ^9-THC	30,000	Negative
11-nor-Δ^9-THC-COOH	1,200	Negative
Verapamil	60,000	Negative
Zomepirac	30,000	Negative

Endogenous, Exogenous Substances and pH Interference

The potential interference from several endogenous and exogenous substances, and pH on the detection accuracy of samples containing amphetamine at ± 50% of the cutoff concentration were tested in the assay. The interfering substances were added to neat oral fluid at the concentrations listed in the table below. The neat oral fluid samples were then processed using the Oral-Eze® collection device and tested in the CEDIA® Amphetamine OFT Assay. No interference was observed with the interfering substances and pH 5-9 samples at the ± 50% cutoff concentrations.

Substances	Tested Concentration in Neat Oral Fluid	Amphetamine OFT Assay	
		-50% Amphetamine	+50% Amphetamine
Low Control	75 ng/mL	Negative	N/A
High Control	225 ng/mL	N/A	Positive
Cotinine	0.03 mg/mL	Negative	Positive
Nicotine	0.03 mg/mL	Negative	Positive
Hemoglobin	0.3 mg/mL	Negative	Positive
Human serum albumin	30 mg/mL	Negative	Positive
Sodium Chloride	18 mg/mL	Negative	Positive

(Con't)

Substances	Tested Concentration in Neat Oral Fluid	Amphetamine OFT Assay	
		-50% Amphetamine	+50% Amphetamine
Cholesterol	0.45 mg/mL	Negative	Positive
Acetaminophen	1.8 mg/mL	Negative	Positive
Acetylsalicylic Acid	1.8 mg/mL	Negative	Positive
Caffeine	0.3 mg/mL	Negative	Positive
Ibuprofen	0.6 mg/mL	Negative	Positive
Coffee	6% v/v	Negative	Positive
Milk	6% v/v	Negative	Positive
Orange Juice	6% v/v	Negative	Positive
Cranberry Juice	6% v/v	Negative	Positive
Soft drink (Coke)	6% v/v	Negative	Positive
Toothpaste	6% v/v	Negative	Positive
Mouthwash	6% v/v	Negative	Positive
Tea	6% v/v	Negative	Positive
Denture Adhesive	6% v/v	Negative	Positive
Alcohol	6% v/v	Negative	Positive
Baking Soda	6% v/v	Negative	Positive
Cough Syrup	6% v/v	Negative	Positive
Whole Blood	6% v/v	Negative	Positive
Hydrogen Peroxide	6% v/v	Negative	Positive
pH	5-9	Negative	Positive

Additional Interference from other Food and Dental Products

Potential interference from additional compounds was tested by collecting neat oral fluid from volunteers after use of the following substances: hard candy, chewing gum, chewing tobacco, cigarettes and tooth whitening strips. The ± 50% controls in the presence of above interfering substances were detected accurately in the CEDIA Amphetamine OFT Assay.

Method Comparison

Forty-two natural (unaltered) neat oral fluid samples from rehabilitation clinics were collected. The neat oral fluid samples were processed using the Oral-Eze collection device. Both the neat and diluted oral fluid samples were tested by LC-MS/MS method while only the diluted samples were tested in the CEDIA Amphetamine OFT Assay.

The overall concordance between the CEDIA Amphetamine OFT Assay and LC-MS/MS using a cutoff of 150 ng/mL in neat oral fluid is 100.0%. The comparison of sample results by the CEDIA Amphetamine OFT Assay to LC-MS/MS showed 100.0% sensitivity and 100.0% specificity.

Candidate Device Results	Less than half the cutoff concentration by LC-MS/MS analysis	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration)	High Positive (greater than 50% above the cutoff concentration)
Negative	18	3	0	0
Positive	0	0	2	19

References

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6. Henderson, D. R., et al. (1986). CEDIA, A New homogeneous Immunoassay System *Clin. Chem.*, 32, 1637-1641.

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