CEDIA® Multi Level THC Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

REF 10016433 (3 x 17 mL Indiko Kit)
100091 (3 x 17 mL Kit)
100100 (65 mL Kit)
1661256 (495 mL Kit)

Intended Use

The CEDIA® THC Assay is an in-vitro diagnostic medical device intended for the qualitative and semiquantitative determination of cannabinoids (THC) in human urine.

The assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Gas chromatography/ mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgement should be applied to any drug of abuse test result particularly when preliminary positive results are used.

Summary and Explanation of Test

Marijuana and hashish come from the hemp plant Cannabis sativa, which grows throughout the world.[2,3] These drugs contain at least 61 cannabinoids (a class of chemicals unique to the cannabis plant), of which Δ^9-tetrahydrocannabinol (THC) is the primary psychoactive compound.[2,3] THC acts as a mild sedative-hypnotic that may produce euphoria, heightened sensations, and, in higher doses, even hallucinations.[2, 4]

THC is highly fat soluble and therefore readily stored in fatty tissues, where it may remain in the body for several days or even weeks.[2,3,5] It is rapidly transformed by liver enzymes to over 24 metabolites, the primary one being 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylicacid.[2-5] Approximately 70% of a THC dose is excreted in feces and urine within 72 hours of administration.[4] The concentrations of THC metabolites in urine are influenced by several factors: the frequency of prior use, the timing of urine specimen collection in relation to the last exposure to THC, and the rate of release of stored cannabinoids from fatty tissues.[5] Heavy chronic THC users who stop taking the drug may show positive urine tests for a month or longer.[2,5]

The CEDIA Multi-Level THC assay uses recombinant DNA technology (US Patent No. 4708929) to produce a unique homogeneous enzyme immunoassay system.[6] This assay is based on the bacterial enzyme β-galactosidase, which has been genetically engineered into two inactive fragments. These fragments spontaneously reassociate to form fully active enzyme that, in the assay format, cleaves a substrate, generating a color change that can be measured spectrophotometrically.

In the assay, drug in the sample competes with drug conjugated to one inactive fragment of β -galactosidase for antibody binding site. If drug is present in the sample, it will bind to antibody, leaving the inactive enzyme fragments free to form active enzyme. If drug is not present in the sample, antibody will bind to drug conjugated on the inactive fragment, inhibiting the reassociation of inactive β-galactosidase fragments, and no active enzyme will be formed. The amount of active enzyme formed and resultant absorbance change are proportional to the amount of drug present in the sample.

Reagents

- **1 EA Reconstitution Buffer:** Contains 3-(N-morpholino) propanesulfonic acid, buffer salts, 0.56 µg/mL monoclonal antibodies to 11-nor-Δ^9-THC-COOH, stabilizer and preservative.
- **1a EA Reagent:** Contains 0.171 g/L Enzyme Acceptor (microbial), buffer salts, detergent and preservative.
- **2 ED Reconstitution Buffer:** Contains 3-(N-morpholino) propanesulfonic acid, buffer salts, stabilizer, and preservative.
- **2a ED Reagent:** Contain 12.42 µg/L Enzyme Donor (microbial) conjugated to 11-nor-Δ^8-THC-COOH, 1.67 g/L chlorophenol red-β-D-galactopyranoside, stabilizer, and preservative.

Additional Materials: Alternative Bar Code Labels (Cat. Nos 100091 and 100100 only. Refer to analyzer specific application sheet for directions on usage). Empty analyzer bottles for EA/ED solution pour-over (Cat. No. 100100) Empty analyzer bottle for ED solution pour-over (Cat. No. 1661256 only).

Additional Materials Required (sold separately):

CEDIA Negative Calibrator
CEDIA THC 25, 50, 75 and 100 Calibrators
THC 25 Control Set , 50 Control Set and 100 Control Set
The calibrators and control set required are dependent on which assay cutoff is being performed. See **Quality Control and Calibration** section for further information.

⚠ Precautions and Warnings

The reagents contain sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10% sodium hydroxide.

Reagent Preparation and Storage

For preparation of the solution for Hitachi analyzers, refer below. For all other analyzers refer to the analyzer-specific application sheet. Remove the kit from refrigerated storage (2-8°C) immediately prior to preparation of the solutions. 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 (15-25°C). Mix again. Record the reconstitution date on the bottle label.

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 (15-25°C) Mix again. Record the reconstitution date on the bottle label.

Cat. No. 100100 - Hitachi 717, 911, 912 or 914 analyzer: Transfer the reconstituted reagents into the corresponding empty R1 and R2 100 mL bottles supplied with the kit. **Hitachi 917 analyzer/ Modular analytics P system:** Use the reconstituted reagents without transfer of bottles.
Cat. No. 1661256 - Hitachi 747 analyzer/Modular analytics D system: Use the funnel provided to transfer a portion of the R2 Solution into the appropriately labeled empty R2 Solution bottle provided.

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 should be yellow-orange in color. A dark red or purple-red 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 sheet for additional information.
NOTE 4: To ensure reconstituted EA solution stability, protect from prolonged, continuous exposure to bright light.

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

Collect urine samples in clean glass or plastic containers. Centrifuge specimens with high turbidity before testing. Human urine should be treated as potentially infectious material. Obtain another sample for testing if adulteration of the sample is suspected. Adulteration of urine samples can affect the test results.

The Mandatory Guidelines for Federal Workplace Drug Testing Programs, Final Guidelines, Notice recommend that specimens that do not receive an initial test within 7 days of arrival at the laboratory should be placed into secure refrigeration units.[7]

Assay Procedure

Chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymtic rates and timing the reaction accurately can be used to perform this assay. Application sheets with specific instrument parameters are available from Microgenics, a part of Thermo Fisher Scientific.

Additional barcode labels are provided for semi-quantitative determination with the 17 mL and 65 mL kits only. To use, over label each bottle with the correct label.

Quality Control and Calibration[8]

Qualitative analysis

For **qualitative analysis** of samples, use the CEDIA THC 25, 50 or 100 ng/mL Calibrator (depending on the selected cutoff), to analyze results. For the Hitachi analyzers, place the selected cutoff calibrator in the appropriate standard position selected by the user. Enter in the S1 ABS as zero and the K factor as 1000 in the Calibration Monitor/Calibration Monitor/Working Information window. For all other analyzers, see the analyzer specific application sheet.

Semiquantitative analysis

For **semiquantitative analysis**, use the THC 50 ng/mL Calibrator together with the Negative, 25, 75 and 100 Calibrators. For Hitachi analyzers, the calibrators are placed in the appropriate standard position selected by the user. For all other analyzers, see the analyzer specific application sheet. Recalibrate the test if reagents are changed or if control results are outside of established limits.

Good laboratory practice suggests that controls be run each day patient samples are tested and each time calibration is performed. It is recommended that two levels of controls be run; one 25% above the selected cutoff; the other 25% below the selected cutoff. Base assessment of quality control on the values obtained for the controls, which should fall within specified limits. If any trends or sudden shifts in values are detected, review all operating parameters. Contact Customer Technical Support for further assistance. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Results and Expected Values

Qualitative results

The CEDIA THC 25, 50 or 100 ng/mL Calibrator is used as a reference in distinguishing between positive and negative samples. Samples producing a response value equal to or greater than the response value of the calibrator are considered positive. Samples producing a response value less than the value of the calibrator are considered negative. Refer to the specific application sheet for additional information.

Semiquantitative results

The CEDIA THC 50 Calibrator, used together with the Negative Calibrator, and the remaining THC Calibrators can be used to estimate relative THC concentrations. Refer to the specific analyzer application sheet for detailed information.

Care should be taken when reporting concentration results since there are many other factors that may influence a urine test result such as fluid intake and other biological factors.

Limitations

1. A positive test result indicates the presence of cannabinoids; it does not indicate or measure intoxication.
2. Other substances and/or factors not listed may interfere with the test and cause false results (eg, technical or procedural errors).

Specific Performance Characteristics

Typical performance data obtained on the Hitachi 717 analyzer are shown below.[9] The results obtained in your laboratory may differ from these data.

Precision

Measured precision studies, using packaged reagents, calibrators, and controls yielded the following results in mA/min with a Hitachi 717 analyzer using NCCLS modified replication experiment guidelines.

25 ng/mL Cutoff	Within-run Precision			Total Precision		
ng/mL	20	25	30	20	25	30
n	120	120	120	120	120	120
$\bar{x}$	373.3	456.14	556.0	373.3	456.1	556.0
SD	3.07	4.67	4.36	13.61	15.20	16.97
%CV	0.9	1.0	0.8	3.6	3.3	3.1

50 ng/mL Cutoff	Within-run Precision			Total Precision		
ng/mL	40	50	60	40	50	60
n	120	120	120	120	120	120
$\bar{x}$	391.2	479.5	584.8	391.2	479.5	584.8
SD	3.20	4.95	5.18	14.41	16.42	17.73
%CV	0.8	1.0	0.9	3.7	3.4	3.0

100 ng/mL Cutoff	Within-run Precision			Total Precision		
ng/mL	75	100	125	75	100	125
n	120	120	120	120	120	120
$\bar{x}$	393.1	505.5	624.7	393.1	505.5	624.7
SD	4.20	6.45	6.05	14.47	16.67	17.99
%CV	1.1	1.3	1.0	3.7	3.3	2.9

Accuracy

Six hundred and thirty-seven urine samples were assayed with the CEDIA THC assay on the Hitachi 717 analyzer using a commercial EIA method as reference. Results were as follows:

A. 25 ng/mL Cutoff

EIA 20ng/mL Cutoff	CEDIA +	CEDIA -
+	163	4*
-	1**	469

B. 50 ng/mL Cutoff

EIA 50ng/mL Cutoff	CEDIA +	CEDIA -
+	159	0
-	1†	477

C. 100 ng/mL Cutoff

EIA 100ng/mL Cutoff	CEDIA +	CEDIA -
+	103	2•
-	18‡	514

* The samples were tested by GC/MS and were found to contain 4-13 ng/mL 11-nor-Δ^9-THC COOH.
** The sample was tested by GC/MS and were found to contain less than 3 ng/mL 11-nor-Δ^9-THC COOH.
† The sample was tested by GC/MS and were found to contain 28 ng/mL 11-nor-Δ^9-THC COOH.
‡ The samples were tested by GC/MS and were found to contain 40-104 ng/mL 11-nor-Δ^9-THC COOH.
• The samples were tested by GC/MS and were found to contain 44-51 ng/mL 11-nor-Δ^9-THC COOH.

Specificity

The following parent compounds and metabolites, when tested with the CEDIA THC assay, yielded the following cross-reactivity results:

Compound	Tested (ng/mL)	% Cross Reactivity
11-nor-Δ^9-THC-COOH	50	100
11-nor-Δ^8-THC-COOH	40	125
Δ^9-THC	500	10.4
11-OH-Δ^9-THC	125	43
8ß-OH-Δ^9-THC	1000	2.8
8ß, 11-di-OH-Δ^9-THC	5000	8.4
1--Δ^9-THC-Glucuronide	62	78
Cannabinol	1000	2.9
Cannabidiol	1000	< 0.1

Structurally unrelated compounds were tested with the CEDIA THC 50 ng/mL cutoff assay, and gave a negative response when tested at the concentrations listed below.

Compound	ng/mL	Compound	ng/mL
Acetaminophen	500,000	Levothyroxine	50,000
Acetylsalicylic acid	500,000	Methadone	500,000
Amoxicillin	100,000	Methamphetamine	500,000
Amphetamine	500,000	Morphine	100,000
Benzoylecgonine	500,000	Nifedipine	500,000
Captopril	500,000	Phencyclidine	500,000
Chlordiazepoxide	100,000	Phenobarbital	500,000
Cimetidine	500,000	Propoxyphene	500,000
Codeine	500,000	Ranitidine	500,000
Diazepam	500,000	Salicyluric acid	500,000
Digoxin	100,000	Secobarbital	500,000
Enalapril	500,000	Tolmetin	500,000
Fluoxetine	500,000	Verapamil	500,000
Ibuprofen	500,000		

Note: A metabolite of the anti-HIV drug Sustiva (formerly known as DMP 266) may cause false positive results in the CEDIA THC assay.

No interference was observed from the following substances added to the normal endogenous concentrations found in urine when tested with the CEDIA THC assay:

Substance	Concentration	Substance	Concentration
Acetone	≤ 1.0 g/dL	Hemoglobin	≤ 0.3 g/dL
Ascorbic acid	≤ 1.5 g/dL	Human serum albumin	≤ 1.0 g/dL
Creatinine	≤ 0.5 g/dL	Oxalic acid	≤ 0.1 g/dL
Ethanol	≤ 1.0 g/dL	Riboflavin	≤ 7.5 mg/dL
Galactose	≤ 10 mg/dL	Sodium Chloride	≤ 6.0 g/dL
γ-globulin	≤ 1.0 g/dL	Urea	≤ 6.0 g/dL
Glucose	≤ 3.0 g/dL		

Sensitivity

For the Qualitative application, the limit of detection (LOD) was 1.3 ng/mL, 2.1 ng/mL and 5.0 ng/mL for the 25 ng/mL, 50 ng/mL and 100 ng/mL cutoff protocols, respectively. For the Semiquantitative application, the LOD was 11.8 ng/mL.

References

1. Hawks, RL. Analytical methodology. In: Hawks, RL, Chiang, CN, eds. Urine Testing for Drugs of Abuse. NIDA Research Monograph 1986, 73:30-41
2. Julien RM. A Primer for Drug Action. 6th ed. New York, NY: WH Freeman & Co; 1992.
3. Chiang CN, Barnett G. Marijuana pharmacokinetics and pharmacodynamics. In: Redda KK, Walker CA, Barnett G, eds. Cocaine, Marijuana, Designer Drugs: Chemistry, Pharmacology, and Behavior. Boca Raton, FL: CRC Press Inc; 1989.
4. Baselt RC, Cravey RH. Disposition of Toxic Drugs and Chemicals In Man. 3rd ed. Chicago, Ill: Year Book Medical Publishers, 1989.
5. Hawks RL, Chiang CN. Examples of specific drug assays. In: Hawks RL, Chiang CN, eds. Urine Testing for Drugs of Abuse. NIDA Research Monograph. 1986; 73: 84-92.
6. Henderson, DR, Friedman, SB, Harris JD. et al. CEDIA, a new homogeneous immunoassay system. Clin Chem. 1986; 32: 1637-1641.
7. Notice of Mandatory Guidelines for Federal Workplace Drug Testing Program: Revised mandatory guidelines, Federal Register. 1994; 110 (June 9): 11983. (Revised Guidelines expected in 2002).
8. Data on traceability are on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
9. Data on file at Microgenics Corporation, a part of Thermo Fisher Scientific.

Microgenics Corporation
46360 Fremont Blvd.
Fremont, CA 94538-6406 USA
US Customer and
Technical Support:
1-800-232-3342

EC REP
Thermo Fisher Scientific Oy
Ratastie 2, P.O. Box 100
01621 Vantaa, Finland
Tel: +358-9-329100
Fax: +358-9-32910300

CE

For insert updates go to:
www.thermoscientific.com/diagnostics

Other countries:
Please contact your local Thermo Fisher Scientific representative.

10006559-2_EN
2012 07

Thermo SCIENTIFIC