CEDIA® UR-144/XLR-11 Assay

Thermo SCIENTIFIC

For Criminal Justice and Forensic Use Only

REF 10022949 (3 x 17 mL Kit)
10022955 (65 mL Kit)

Intended Use

The CEDIA® UR-144/XLR-11 Assay is a homogenous enzyme immunoassay for the qualitative and semi-quantitative determination for the presence of UR-144/XLR-11 and their metabolites in human urine at a cutoff concentration of 10 ng/mL. The assay is intended to be used in laboratories and provides a simple and rapid analytical screening procedure to detect UR-144/XLR-11 and their metabolites in human urine.

The assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used in order to confirm an analytical result. Gas chromatography/mass spectrometry (GC/MS) and Liquid Chromatography/tandem mass spectrometry (LC-MS/MS) are the preferred confirmatory methods.[1]

The semi-quantitative mode is for the purpose of detection and enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as Liquid chromatography/tandem mass spectrometry (LC-MS/MS) or permitting laboratories to establish quality control procedures.

Clinical and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used. For Criminal Justice and Forensic Use Only.

Summary and Explanation of the Test

Synthetic cannabinoids are a class of synthetic compounds chemically similar to THC, the psychoactive ingredient in marijuana. Synthetic cannabinoids interact CB1 and CB2 receptors like THC[2], therefore causing the same physiological effects, but often with higher potency and higher health risks to the users. Synthetic cannabinoids are typically sprayed on a mixture of shredded plant material and marketed as incense that can be smoked. Short term effects of synthetic cannabinoids include loss of control, lack of pain response, increased agitation, pale skin, seizures, vomiting, profuse sweating, uncontrolled/spastic body movements, elevated blood pressure, heart rate and palpitations.[2,3] In addition to physical signs, users may experience dysphoria, severe paranoia, delusions, hallucinations and increased agitation. UR-144 and XLR-11 are among the commonly used synthetic cannabinoid drugs. Major metabolites of UR-144 and XLR-11 detectable in human urine samples include UR-144 N-pentanoic acid and UR-144 5-hydroxypentyl.[4, 5]

The CEDIA UR-144/XLR-11 Assay uses recombinant DNA technology to produce a unique homogeneous enzyme immunoassay system.[6] The assay is based on the bacterial (*Escherichia coli*) enzyme β-galactosidase, which has been genetically engineered into two inactive fragments (enzyme donor and enzyme acceptor). These fragments spontaneously re-associate to form fully active enzyme that, in assay format, cleave a substrate, generating color change that can be measured spectro-photometrically.

In this assay, the analyte in the specimen competes with the analyte conjugated to enzyme donor (ED) for antibody binding sites. If the analyte is present in the sample, it binds to the antibody, leaving the ED conjugate free to form active enzyme with the enzyme acceptor (EA). If the analyte is not present in the sample, antibody binds to the analyte conjugated to ED, inhibiting the re-association of ED to EA, and no active enzyme is formed. The amount of active enzyme formed and resultant absorbance change are directly proportional to the amount of drug present in the sample.

Reagents

1 EA Reconstitution Buffer
Contains buffer salts, mouse monoclonal anti-UR-144 antibody 0.1 mg/L, stabilizer, and preservative.

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

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

2a ED Reagent
Contains 0.175 mg/L Enzyme Donor, conjugated to UR-144 derivative, 1.67 g/L chlorophenol red-β-D-galactopyranoside, stabilizers, detergent and preservative.

Additional Materials (sold separately):

REF	Kit Description
10022753	CEDIA Negative Calibrator II (1 x 7.5 mL)
10022754	CEDIA UR-144 10 ng/mL Calibrator (1 x 5 mL)
10022755	CEDIA UR-144 20 ng/mL Calibrator (1 x 5 mL)
10022756	CEDIA UR-144 40 ng/mL Calibrator (1 x 5 mL)
10022759	CEDIA UR-144 60 ng/mL Calibrator (1 x 5 mL)
10022760	CEDIA UR-144 Control Set (5 ng/mL and 15 ng/mL, 2 x 5 mL each)

⚠ Warnings and Precautions

The reagents are harmful if swallowed.

DANGER: The powder reagents contain ≤55% w/w Bovine Serum Albumin (BSA) fragment and ≤1% w/w Sodium Azide. Liquid reagents contain ≤0.5% Bovine Serum, ≤0.2% 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 mist or vapor. 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.

In the case of accidental spill, clean and dispose of material according to your laboratory's Standard Operating Procedure (SOP), local, and state regulations.

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

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. Do not use the reagents beyond the expiration dates.

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. Discard reagents if turbidity or precipitates are observed.

⚠ **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 shelf life of the unopened components, refer to the box or bottle labels for the expiration date.

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

Specimen Collection and Handling

Collect urine specimens in plastic or glass containers. Care should be taken to preserve the chemical integrity of the urine sample from the time it is collected until the time it is assayed.

Specimens kept at room temperature that do not receive initial test within 7 days of arrival at the laboratory should be placed into a secure refrigeration unit at 2 to 8°C for up to 12 weeks.[7,8] For longer storage prior to analysis or for sample retention after analysis, urine specimens may be stored at -20°C.[8]

Laboratories following the SAMHSA mandatory guidelines should refer to SAMHSA "Short-Term Refrigerated Storage" and "Long-Term Storage" requirements.[7]

To protect the integrity of the sample, do not induce foaming and avoid repeated freezing and thawing. An effort should be made to keep pipetted samples free of gross debris. It is recommended that grossly turbid specimens be centrifuged before analysis. Frozen samples should be thawed and mixed prior to analysis. Adulteration of the urine sample may cause erroneous results. If adulteration is suspected, obtain another sample and forward both specimens to the laboratory for testing.

Handle all urine specimens as if they were potentially infectious.

Assay Procedure

The CEDIA UR-144/XLR-11 Assay is intended for use on automated clinical chemistry analyzers capable of maintaining a constant temperature, pipetting, mixing reagents, measuring enzymatic rates at 570 nm and timing the reaction accurately.

Refer to specific application instructions for each analyzer for chemistry parameters before performing the assay.

Qualitative analysis

For qualitative analysis, use the CEDIA UR-144 10 ng/mL Calibrator as the cutoff level.

Semi-quantitative analysis

For semi-quantitative analysis, use all five calibrators.

Quality Control and Calibration

Good laboratory practice requires the use of control specimens to ensure proper assay performance. Ensure that control results are within the established ranges, as determined by laboratory procedures and guidelines. If results fall outside of the 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. Each laboratory should establish its own quality control testing frequency.

Results and Expected Values

Qualitative

The 10 ng/mL calibrator is used as a cutoff reference for distinguishing 'positive' from 'negative' samples. A sample that exhibits a change in absorbance values (ΔA) equal to or greater than that obtained with the cutoff calibrator is considered as positive. A sample that exhibits a change in absorbance value (ΔA) lower than that obtained with the cutoff calibrator is considered as negative.

Semi-quantitative

An estimate of drug concentrations in the samples can be obtained by running a standard curve with all calibrators and estimating sample concentrations off the standard curve. Sample results above the high calibrator should be diluted with negative calibrator and retested.

Limitations

1. A positive result from this assay indicates the presence of UR-144/XLR-11 and other structurally related compounds, and does not necessarily correlate with the extent of physiological and psychological effects. This is a screening test. All positive results must be confirmed via GC/MS or LC-MS/MS.
2. It is possible that substances other than those investigated in the specificity study may interfere with the test and cause false results.
3. Care should be taken when reporting concentration results since there are many factors e.g. fluid intake and other biologic factors that may influence a urine test result.
4. Performance characteristics for the CEDIA UR-144/XLR-11 assay performance have not been established with body fluids other than human urine.

Specific Performance Characteristics

Typical performance results obtained on Olympus AU680 analyzer (Beckman Coulter) are shown below. The results obtained in your laboratory may differ from these data.

Precision

Samples were prepared by spiking the calibrator analyte into drug free urine at the cutoff (10 ng/mL), 5 ng/mL and 15 ng/mL, and tested in both qualitative and semi-quantitative modes using a modified Clinical Laboratory and Standards Institute (CLSI) protocol. Results presented below were generated by testing the samples in replicates of 6, twice per day for 5 days, total n=60.

Qualitative Study Analysis

Conc. (ng/mL)	% of cutoff (10 ng/mL)	Total Precision (n=60)	
		Number of Determinations	Immunoassay Results (Negative/Positive)
5	-50%	60	60/0
15	+50%	60	0/60

Semi-Quantitative

Conc. (ng/mL)	% of cutoff (10 ng/mL)	Precision (n=60)	
		Within Run CV	Total CV
5	-50%	9.92%	13.96%
10	100%	6.30%	7.04%
15	+50%	6.08%	7.05%

Accuracy

A total of eighty-four urine samples were analyzed by the CEDIA UR-144/XLR-11 Assay in qualitative and semi-quantitative modes and the results were compared to LC-MS/MS.

At 10 ng/mL cutoff, the CEDIA UR-144/XLR-11 Assay tested 31 of the 34 positive urine samples confirmed by LC-MS/MS as positive, and all 50 of the confirmed negative samples as negative.

		LC-MS/MS +	LC-MS/MS -
CEDIA UR-144/XLR-11 Assay (cutoff 10 ng/mL)	+	31	0
	-	3*	50

* Samples were detected between 7 and 9 ng/mL.

Specificity

The cross-reactivity of UR-144/XLR-11, their metabolites, and other cannabinoids was evaluated by adding known amounts of each metabolite into drug free urine.

The following table lists cross-reactivity of compounds structurally related to UR-144 (including UR-144 metabolites, derivatives, additional synthetic cannabinoids, and natural cannabinoids) using the cutoff of 10 ng/mL.

Synthetic Cannabinoids Compounds	Tested Concentration (ng/mL)	Observed Concentration (SQ Conc) (ng/mL)	% Cross-reactivity
UR-144 N-(5-hydroxypentyl) β-D- glucuronide	5	12	240%
UR-144 N-(5-hydroxypentyl)	5	11	220%
UR-144 N-pentanoic acid	10	12	120%
XLR-11	15	15	100%
XLR-11 N-(4-pentenyl) analog	15	14	93%
UR-144 N-(4-hydroxypentyl)	15	11	73%
UR-144	20	11	55%
XLR-11 N-(2-fluoropentyl) isomer	20	11	55%
XLR-12	25	11	44%
UR-144 N-(2-hydroxypentyl)	35	11	31%
UR-144 N-(5-chloropentyl) analog	50	14	28%
UR-144 N-(5-bromopentyl) analog	100	17	17%
A-834,735	100	12	12%
UR-144 N-heptyl analog	100	12	12%
A-836,339	100	12	12%
AM-2201 N-(4-hydroxypentyl)	250	7	2.8%
AKB48 N-(5-hydroxypentyl)	300	8	2.67%
5-Fluoro SDB-006	500	6	1.2%
JWH-018 N-pentanoic acid	500	6	1.2%
UR-144 Degradant	500	1	0.2%
AKB48 N-pentanoic acid	500	3	0.6%
AKB48 N-(4-hydroxypentyl)	500	2	0.4%
5-Fluoro AKB48 N-(4-hydroxypentyl)	1,000	3	0.3%
FUB-144	1,000	2	0.2%
UR-144 desalkyl	1,000	2	0.2%
JWH-250 N-(5-hydroxypentyl)	1,000	6	0.6%
AB-005	1,000	1	0.1%
A-796260	1,000	8	0.8%
JWH-018 N-(5-hydroxypentyl)	1,000	4	0.4%
JWH-018 N-(4-hydroxypentyl)	1,000	4	0.4%

Table continued

Synthetic Cannabinoids Compounds	Tested Concentration (ng/mL)	Observed Concentration (SQ Conc) (ng/mL)	% Cross-reactivity
JWH-018 N-(5-hydroxypentyl) β-D-glucuronide	1,000	7	0.7%
AM-1220	1,000	8	0.8%
JWH-073 N-(5-hydroxybutyl)	1,000	3	0.3%
JWH-073 N-butanoic acid	1,000	7	0.7%
JWH-200	1,000	5	0.5%
Delta 9-THC	1,000	2	0.2%
AB-PINACA N-pentanoic acid	10,000	2	0.02%
ADB-PINACA N-pentanoic acid	10,000	2	0.02%
AM-694	10,000	6	0.06%
AM-2233	10,000	3	0.03%
JWH-019	10,000	3	0.03%
JWH-022	10,000	2	0.02%
JWH-020	10,000	2	0.02%
PB-22 N-pentanoic acid	10,000	8	0.08%
PB-22 N-pentanoic acid 3-carboxyindole	10,000	1	0.01%
PB-22 3- carboxyindole	10,000	2	0.02%
FUB-PB-22	10,000	1	0.01%
FDU-PB-22	10,000	2	0.02%
(-) - CP - 55,940	10,000	1	0.01%
(+)- CP- 55,940	10,000	2	0.02%
BB-22	10,000	8	0.08%
MDMB-CHIMICA	10,000	2	0.02%
CD-47,497-C8	10,000	3	0.03%

The potential cross-reactivity posed by drugs commonly co-administered was evaluated by adding each substance to drug free urine at the concentration indicated. A drug was considered to cross-react if the observed UR-144 concentrations result exceeded 10 ng/mL. As shown in the tables below, all the pharmacologic compounds evaluated, including a number of opiate compounds, exhibited no cross reactivity at the concentrations tested.

Opiate compounds produced a negative result at the concentrated listed below

Opiate Compounds	Tested Concentration (ng/mL)
6-Acetyl Morphine	100,000
Diacetylmorphine (Heroin)	100,000
Codeine	100,000
Codeine β-D-glucuronide	75,000
Dihydrocodeine	100,000
Dextromethorphan	100,000
EDDP	100,000
EMDP	50,000
Fentanyl	100,000
Hydrocodone	100,000
Hydromorphone	100,000
Hydromorphone β-D-glucuronide	100,000
Methadone	100,000
Meperidine	100,000
Morphine	100,000
Morphine 3-β-D-glucuronide	100,000
Morphine 6-β-D-glucuronide	100,000
Nalorphine	100,000
Naloxone	100,000
Naltrexone	50,000

Table continued

Opiate Compounds	Tested Concentration (ng/mL)
Norcodeine	100,000
Norhydrocodone	100,000
Noroxycodone	100,000
Noroxymorphone	100,000
Norpropoxyphene	100,000
Oxymorphone 3-β-D-glucuronide	100,000
Oxycodone	100,000
Oxymorphone	100,000
Tapentadol	100,000
Tramadol	100,000

Structurally unrelated compounds and other concomitantly used drugs produced a negative result at the concentrations listed below

Compounds	Tested Concentration (ng/mL)
Acetaminophen	500,000
Acetylsalicylic acid	500,000
Amitriptyline	100,000
Amoxicillin	100,000
Amphetamine	1,000,000
Atropine	10,000
Benzoylecgonine	1,000,000
Cannabicyclol	10,000
Cannabidiol	100,000
Caffeine	100,000
Carbamazepine	100,000
Chloroquine	100,000
Chlorpromazine	100,000
Clomipramine	250,000
Cimetidine	500,000
Desipramine	100,000
Doxepin	100,000
Diphenhydramine	100,000
Efavirenz	10,000
Ephedrine	1,000,000
Fluoxetine	100,000
Fluphenazine	100,000
Hydroxychloroquine	100,000
11- Hydroxy-THC	10,000
Ibuprofen	100,000
Imipramine	100,000
Nalbuphine	100,000
11-nor-9- Carboxy-THC	10,000
Nortriptyline	100,000
Oxazepam	100,000
Phenobarbital	100,000
Psilocybin	10,000
Ranitidine	500,000
Scopolamine	10,000
Thioridazine	100,000
Trimipramine	250,000

Interference

The potential interference of pH and endogenous physiologic substances on recovery of UR-144 controls using CEDIA UR-144/XLR-11 Assay was assessed by spiking known compounds of potentially interfering substances into the low (5 ng/mL) and high (15 ng/mL) controls for 10 ng/mL cutoff. No interference was observed with urine samples containing the compounds at the concentrations listed below.

Compounds	Tested Concentration (mg/dL)	Spiked calibrator analyte level	
		Low Control	High Control
Acetaminophen	10	NEG	POS
Acetone	500	NEG	POS
Acetyl Salicylic Acid	10	NEG	POS
Ascorbic Acid	150	NEG	POS
Caffeine	10	NEG	POS
Creatinine	400	NEG	POS
Ethanol	100	NEG	POS
Galactose	10	NEG	POS
Glucose	1,000	NEG	POS
Hemoglobin	150	NEG	POS
Human Serum Albumin	200	NEG	POS
Ibuprophen	10	NEG	POS
Oxalic acid	50	NEG	POS
Riboflavin	5	NEG	POS
Sodium Chloride	2,000	NEG	POS
Urea	2,000	NEG	POS
pH (5 to 9)	NA	NEG	POS

References

1. *Mandatory Guideline for Federal Workplace Drug Testing Programs*. National Institute on Drug Abuse. Federal Register Vol. 73, No. 228, 2008:71893.
2. UR-144 Critical-Review Report, Agenda item 4.8, WHO Expert Committee on Drug Dependence 36th meeting. June 2014.
3. Jordan Trecki, et al., Synthetic Cannabinoid-Related Illnesses and Deaths N ENGL J MED 2015; 373(2): 103 - 107.
4. Nielsen LM, et al., Cytochrome P450-mediated metabolism of the synthetic cannabinoids UR-144 and XLR-11. Drug Testing and Analysis 2016; 8(8): 792 - 800.
5. Wohlfarth, A, et al., First metabolic profile of XLR-11, a novel synthetic cannabinoid, obtained by using human hepatocytes and high-resolution mass spectrometry. Clin. Chem. 2013; 59(11): 1638 – 1648.
6. Henderson D, et al., CEDIA, A new homogenous immunoassay system. Clin. Chem. 1986; 32(9): 1637 – 1641.
7. *Notice of Mandatory Guidelines for Federal Workplace Drug Testing Program: Final Guidelines; Federal Register,* Substance Abuse and Mental Health Administration (SAMHSA), 1994; 110 (June 9):11983.
8. "Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline", Second Edition, C52-A2, Clinical and Laboratory Standards Institute, April 2007.

Key to Symbols Used


Manufacturer


Caution

Lot Number/Batch Code


Use-by Date


Consult Instructions for Use

Catalog Number


Temperature Limit


Health Hazard

Reagents

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10022961-0-EN
2017 05

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