MEDICA

IVD CE

REF 14242-4 4 X 24 mL/9 mL

CANNABINOID ENZYME IMMUNOASSAY (THC)
Wedges each contain usable volumes of 24 mL of R1 reagent and 9 mL of R2 reagent.

INTENDED USE

The EasyRA Cannabinoid (THC) reagent is intended for the qualitative determination of cannabinoids in human urine using 11-nor-Δ^9-THC-9-COOH, (the major metabolite of THC, referred to hereafter as cTHC) at a cutoff value of 50 ng/mL. The assay is designed for prescription use on the EasyRA Clinical Chemistry Analyzer.

The assay provides a rapid screening procedure for determining the presence of cannabinoids in urine. The assay provides only a preliminary analytical result. A more specific alternative analytical chemistry method must be used in order to obtain a confirmed analytical result. Gas or Liquid Chromatography/Mass Spectrometry (GC/MS or LC/MS) are the preferred confirmatory methods (1, 2). Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.

SUMMARY AND EXPLANATION OF TEST

The principal, active constituent in marijuana or hashish, obtained from the *Cannabis sativa* plant, is Δ^1-3, 4-*trans* tetrahydrocannabinol, frequently referred to as Δ^9-tetrahydocannabinol or Δ^9-THC. Cannabis has been used for its euphoric effects for over 4000 years (3). It is one of the most commonly used drugs in the United States.

Marijuana is frequently self-administered for its mood-altering properties. Chronic use has been shown to cause reversible psychological impairment, abstinence syndrome, and development of tolerance (4). At low dose, it produces mixed depressant and stimulant effects; at higher dose, marijuana acts as a CNS depressant (5-7).

In the past few decades, research has confirmed the presence of an endogeneous endocannabinoid system or ECS (8). Endocannabinoids are produced within the human body and activate two known cannabinoid receptors, CB_1 and CB_2 (9). The CB_1 receptor is localized primarily to the brain and is thought to be responsible for the euphoric and anticonvulsive effects of cannabis, whereas the CB_2 receptor is found primarily in the immune system and thought to be responsible for the anti-inflammatory effects of Δ^9-THC (10-12).

Studies published in 2006 revealed that Δ^9-THC may actually induce cell death (13) and may even be a viable anti-tumor target (14). Due to the role the ECS may play in a number of physiological processes, much interest in the use of synthetic ECS ligands for therapeutic purposes remains high (15-17).

Δ^9-THC is easily absorbed by inhalation (smoking) or ingestion. Due to its highly fat-soluble nature, Δ^9-THC is readily deposited in fatty tissues, where it may remain for days or even weeks (5). It is primarily metabolized in the liver to a variety of compounds, the major one being the cTHC metabolite (6, 7). Approximately 70 % of THC is excreted in feces and urine within 72 hours of administration (18).

Assay Principle

The EasyRA Cannabinoid (THC) reagent is a homogeneous enzyme immunoassay with ready-to-use liquid reagent. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent (19). Enzyme activity decreases upon binding to the antibody, and the drug concentration in the sample is measured in terms of enzyme activity. In the absence of drug in the sample, cannabinoid derivative-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when drug is present in the sample, antibody binds to free drug; the unbound cannabinoid derivative-labeled G6PDH then exhibits its maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at a 340 nm primary wavelength.

REAGENTS PROVIDED

Antibody/Substrate Reagent (R_1): Contains mouse monoclonal anti-cannabinoid antibody, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD), and sodium azide (0.09 %) as a preservative.

Enzyme-drug Conjugate Reagent (R_2): Contains cannabinoid derivative-labeled glucose-6-phosphate dehydrogenase (G6PDH) in buffer with sodium azide (0.09 %) as a preservative.

Precautions and Warnings

- *This test is for in vitro diagnostic use only. Harmful if swallowed.*
- *Reagent contains sodium azide as a preservative, which may form explosive compounds in metal drain lines. When disposing such reagents or wastes always flush with a large volume of water to prevent azide build-up. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (8/16/76).*
- *Do not use the reagents beyond their expiration dates.*

Instructions for Reagent Handling, Storage & Stability

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date on the label if stored at 2–8ºC. Opened reagent is stable on-board in the refrigerated reagent area of the Medica EasyRA Chemistry Analyzer for the number of days programmed on the RFID chip on the reagent wedge. Remove the cap and place the reagent in the Medica EasyRA Chemistry Analyzer reagent tray located in the reagent area.

SPECIMEN COLLECTION AND HANDLING

Urine samples may be collected in plastic or glass containers. Some plastics may adsorb drugs (20-22). Use of plastics such as polyethylene is recommended (23). Use fresh urine specimens for the test. If a sample cannot be analyzed immediately, it may be refrigerated at 2–8ºC for up to three days. For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown Δ^9-THC analytes in urine are stable at -20ºC for up to 40 days (24–26). Samples should be at room temperature (18–25ºC) for testing. Samples with high turbidity should be centrifuged before analysis.

Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and forward both samples to the laboratory for testing. *Handle all urine specimens as if they are potentially infectious.*

PROCEDURE

Materials Provided

Medica THC Reagent Wedge, REF 14242 (Qualitative)

Additional Materials Required

Medica EasyCal THC Cutoff Calibrator (THC Cutoff, 50 ng/mL), REF 14659
Medica EasyCal THC Negative Calibrator (THC, 0 ng/mL), REF 14800
Medica EasyQC THC Negative Control (THC, 37.5 ng/mL), REF 14778
Medica EasyQC THC Positive Control (THC 62.5 ng/mL), REF 14779
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 or
Medica Cleaner Wedge – Chemistry, REF 10661
Medica EasyRA Evaporation Caps, REF 10745

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzyme rates at a 340 nm primary wavelength and timing the reaction accurately can be used to perform this homogeneous immunoassay.

Performance characteristics presented in this package insert have been validated on the Hitachi 717 and on the EasyRA Clinical Chemistry Analyzer.

Assay Procedure

Analyzers with the specifications indicated above are suitable for performing this homogeneous enzyme immunoassay. Refer to the specific parameters used for each analyzer before performing the assay. For qualitative analysis use the 50 ng/mL as the cutoff calibrator. Recalibration should be performed if there is a change in calibrators or reagent lot. Two levels of controls are also available for monitoring of the cutoff level: use the 37.5 ng/mL and 62.5 ng/mL for the 50 ng/mL cutoff level.

INSTRUCTIONS FOR USE

The reagent is ready to use as supplied. Remove the cap and place the reagent in the Medica EasyRA Chemistry Analyzer reagent tray in the reagent area. Dry the neck of the reagent wedge and check the inside of the necks of the wedge for foam after removing the caps and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2. Place Medica EasyRA Evaporation Caps, REF 10745 on both the R1 and R2 openings of the reagent wedge.
NOTE: Use of the Medica EasyRA Evaporation Cap is required to guarantee on-board calibration stability.

Calibration

Medica EasyCal THC Cutoff Calibrator, REF 14659 and Medica EasyCal THC Negative Calibrator, REF 14800 are required for the calibration of the assay. The calibration interval (30 days maximum) with evaporation caps is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

Quality Control

Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument system manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters or contact Medica Corporation technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

Results

Note: A positive test result does not always mean a person took illegal drugs and a negative test result does not always mean a person did not take illegal drugs. There are a number of factors that influence the reliability of drug tests.

Qualitative: The cutoff calibrator, which contains 50 ng/mL of cTHC, is used as a reference for distinguishing positive from negative samples. A sample with a change in absorbance (ΔmA/min) greater than that obtained with the cutoff calibrator is considered positive. A sample with a change in absorbance (ΔmA/min) equal to or lower than that obtained with the cutoff calibrator is considered negative.

Procedural Limitations

1. A positive result from the assay indicates only the presence of cannabinoids.
2. The test is not intended for quantifying these single analytes in samples.
3. A positive result does not necessarily indicate drug abuse.
4. A negative result does not necessarily mean a person did not take illegal drugs.
5. Care should be taken when reporting results, as numerous factors (e.g., fluid intake, endogeneous or exogeneous interferents) may influence the urine test result.
6. Positive results should be confirmed by other affirmative, analytical chemistry methods (e.g., chromatography), preferably GC/MS or LC/MS.
7. The test is designed for use with human urine only.
8. The test is not for therapeutic drug monitoring.

Typical Performance Characteristics

The results shown below were performed with a single Hitachi 717 automated clinical chemistry analyzer and validated on an EasyRA Clinical Chemistry Analyzer.

Precision:

Qualitative analysis: The five calibrators were evaluated. Typical results (ΔmA/min) are as follows:

N=88 (mA/min)	Within Run			Run-to-Run		
	Mean	SD	% CV	Mean	SD	% CV
0 ng/mL	387.3	3.0	0.8 %	387.3	3.8	1.0 %
12.5 ng/mL	410.9	2.5	0.6 %	410.9	3.9	0.9 %
25.0 ng/mL	438.8	2.8	0.6 %	438.8	3.9	0.9 %
37.5 ng/mL	465.7	2.6	0.6 %	465.7	3.9	0.8 %
50.0 ng/mL	503.3	2.9	0.6 %	503.3	4.2	0.8 %
62.5 ng/mL	536.3	2.8	0.5 %	536.3	3.6	0.7 %
75.0 ng/mL	559.0	3.9	0.7 %	559.0	4.7	0.8 %
87.5 ng/mL	590.2	2.6	0.4 %	590.2	4.1	0.7 %
100.0 ng/mL	603.8	2.5	0.4 %	603.8	3.8	0.6 %

Results (cont'd)

50 ng/mL Cutoff Result:		Within Run		Total Precision	
Sample [ ] (ng/mL)	**% of Cutoff**	**# Samples**	**EIA Result**	**# Samples**	**EIA Result**
0 ng/mL	0 %	22	22 Neg	88	88 Neg
12.5 ng/mL	25 %	22	22 Neg	88	88 Neg
25.0 ng/mL	50 %	22	22 Neg	88	88 Neg
37.5 ng/mL	75 %	22	22 Neg	88	88 Neg
50.0 ng/mL	100 %	22	5 Pos/ 17 Neg	88	32 Pos/ 56 Neg
62.5 ng/mL	125 %	22	22 Pos	88	88 Pos
75.0 ng/mL	150 %	22	22 Pos	88	88 Pos
87.5 ng/mL	175 %	22	22 Pos	88	88 Pos
100.0 ng/mL	200 %	22	22 Pos	88	88 Pos

Sensitivity: Sensitivity, defined as the lowest concentration that can be differentiated from the negative urine with 95 % confidence, was tested to be 10 ng/mL.

Accuracy: Sixty-eight (68) negative and sixty-five (65) positive specimens for a total of one-hundred-thirty-three (133) unaltered clinical urine specimens were tested at the 50 ng/mL cutoff and confirmed with GC/MS or LC/MS. Specimens having a concentration of cTHC greater than 50 ng/mL by GC/MS or LC/MS were defined as positive, and specimens with lower concentrations by GC/MS or LC/MS were defined as negative in the tables below. Near cutoff samples are defined as ± 50 % of the cutoff value. The correlation results are summarized as follows:

THC 50 - Qualitative Accuracy Study:

50 ng/mL Cutoff	Neg	<50 % of the cutoff	Near Cutoff Neg	Near Cutoff Pos	> 50 % above the cutoff	% Agreement
Positive	0	1*	6**	15	50	100.0 %
Negative	11	35	15	0	0	89.7 %

Summary of Discordant Results in Qualitative Mode :

	Cutoff Value	cTHC EIA (Pos/Neg)	cTHC GCMS Value (ng/mL)
Qualitative Mode	**50 ng/mL**	**Positive**	**7**[∂]
		Positive	**27**
		Positive	**44.7**
		Positive	**46**
		Positive	**48**
		Positive	**48**
		Positive	**49.3**

[∂] *The discrepant result showing a GCMS value of 7 ng/mL was initially obtained in our first method comparison study. Due to the small sample size received, we were unable to further retest this sample. Following additional method comparison studies, we believe the root cause of the error was derived from initial GCMS readings. The additional method comparison studies and initial studies have been combined in the accuracy report listed above.*

Analytical Recovery: To demonstrate linearity for purposes of sample dilution and quality control, a drug-free urine pool was spiked with cTHC and serially diluted. Each sample was run in 10 replicates and the average was used to determine the functional linearity range of the assay. When comparing the result (y) and target (x) value, using the least squares regression technique, the regression equation and correlation are as follows:

$y = 1.029x - 0.0268$, $r^2 = 0.9989$

% Dilution	Target Concentration (ng/mL)	Determined (ng/mL)	% Recovery
100 %	0	0.4	N/A
90 %	10	11.3	112.9 %
80 %	20	20.2	101.1 %
70 %	30	29.6	98.5 %
60 %	40	39.2	98.0 %
50 %	50	51.6	103.2 %
40 %	60	62.2	103.7 %
30 %	70	74.0	105.7 %
20 %	80	82.7	103.4 %
10 %	90	92.9	103.3 %
0 %	100	101.6	101.6 %

Specificity

Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine calibrator matrix individually to various concentrations and evaluated against the cutoff calibrator.

The table listed the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator (as positive) or the concentration of the compounds tested that gave a response below the response of the cutoff calibrator (as negative).

Structurally Related Cannabinoids (cTHC) Compounds:

Compound	Target (ng/mL)	EIA (ng/mL)	% Cross Reactivity
8-β-hydroxy- Δ^9-THC	80	46.4	58.0%
8-β-11-dihydroxy- Δ^9-THC	90	40.9	45.4%
Cannabidiol	9,000	46.1	0.5%
Cannabinol	220	46.4	21.1%
exo-THC	90	46.8	52.0%
ƒ-11-Hydroxy- Δ^9-THC	55	52.8	96.0%
ƒ-11-Nor-Δ^9-THC-9-Carboxylic Acid	50	49.4	98.8%
ƒ-11-Nor-Δ^9-THC-9-Carboxylic Acyl-Glucuronide	5,000	70.4	1.4%
Δ^8-THC	90	46.6	51.8%
Δ^9-THC	140	46.4	33.1%

Structurally Unrelated Pharmacological Compounds:

Compound	Target (ng/mL)	EIA (ng/mL)	% Cross Reactivity
Acetaminophen	500,000	3.9	0.001%
Acetylsalicylic acid	500,000	2.0	0.000%
Amitryptyline	500,000	2.2	0.000%
Amobarbital	500,000	0.9	0.000%
Amphetamine	500,000	1.3	0.000%
Benzoylecgonine	500,000	4.8	0.001%
Bupropion	500,000	3.1	0.001%
Caffeine	500,000	4.9	0.001%
Chlorpheniramine	500,000	5.2	0.001%
Chlorpromazine	500,000	2.6	0.001%
Cocaine	500,000	2.3	0.000%
Codeine	500,000	2.4	0.000%
Dextromethorphan	500,000	2.2	0.000%
Ecgonine Methyl Ester	500,000	2.9	0.001%
d,l-Ephedrine	500,000	2.2	0.000%
Imipramine	500,000	1.3	0.000%
JWH-018(l-pentyl-3(1-naphthoyl)indole)	500,000	7.3	0.001%
JWH-073(1-butyl-3(1-naphthoyl)indole)	500,000	6.7	0.001%

(con't'd) Compound	Target (ng/mL)	EIA (ng/mL)	% Cross Reactivity
Lidocaine	500,000	5.7	0.001%
Meperidine	500,000	2.1	0.000%
Methadone	500,000	3.6	0.001%
Methamphetamine	500,000	2.0	0.000%
Methaqualone	500,000	6.8	0.001%
Morphine	500,000	1.3	0.000%
Nortriptyline	500,000	1.0	0.000%
Oxazepam	500,000	3.0	0.001%
Phencyclidine	500,000	1.5	0.000%
Phenobarbital	500,000	4.5	0.001%
Promethazine	500,000	4.1	0.001%
Propoxyphene	500,000	3.7	0.001%
Ranitidine	500,000	4.9	0.001%
Secobarbital	500,000	3.7	0.001%
Valproic Acid	500,000	5.5	0.001%

It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results

Interference

The following endogenous compounds were spiked into a pool of processed negative urine (cannabinoids free urine) to the desired concentrations listed in the table below. Standards of cTHC were then spiked into the pools of processed urine containing the endogenous compounds to the concentrations listed below as positive or negative controls. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding positive/negative results against the cutoff values of 50 ng/mL. Results are summarized in the following table:

Interfering Substances	Spiked (mg/dL)	0 ng/mL	37.5 ng/mL Control	62.5 ng/mL Control
None		1.5	33.8	59.3
Acetone	1000	3.1	32.5	57.5
Ascorbic Acid	500	0.0	33.4	55.0
Creatinine	500	1.4	33.1	56.5
Ethanol	1000	5.2	34.9	58.4
Galactose	10	1.9	36.2	60.0
Y-Globulin	500	2.1	29.9	53.7
Glucose	1500	2.1	34.4	59.1
Hemoglobin	300	4.5	33.7	60.1
Human Serum Albumin	500	5.6	33.9	60.6
Oxalic Acid	100	0.9	32.7	51.8
Riboflavin	0.65	0.0	35.0	59.2
Sodium Chloride	2000	0.0	33.4	56.5
Urea	2000	2.0	32.8	59.1
pH 3	N/A	0.0	30.3	50.6
pH 4	N/A	0.0	33.8	56.8
pH 5	N/A	0.0	35.6	60.9
pH 6	N/A	1.2	35.9	64.5
pH 7	N/A	4.2	37.1	67.6
pH 8	N/A	3.6	39.4	67.0
pH 9	N/A	4.6	39.8	67.5
pH 10	N/A	3.9	40.2	71.0
pH 11	N/A	5.3	34.8	63.7

Specific Gravity: Urine samples with specific gravity values ranging from 1.002 to 1.025 were tested with the assay in the presence of 0 ng/mL, 37.5 ng/mL, and 62.5 ng/mL (positive and negative controls for THC 50) of cTHC, and no interference was observed.

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EasyRA Clinical Chemistry Analyzer

Parameters:	**Qualitative**
Primary Wavelength	340
Secondary Wavelength	N/A
Reaction Type	Qual. Kinetic
Reaction Direction	Increase
Calibration Curve	Increase
Reagent Blank	N/A
Sample Blank	N/A
Reaction Time	4.0 Minutes
On-Board Stability	30 Days
Cal Stability	13 Days*

*with evaporation caps

Performance Characteristics

The results shown below were obtained with the EasyRA analyzer.

Inaccuracy/Correlation

Eighty (80) clinical urine specimens were tested qualitatively with the Enzymatic Immunoassay (EIA) method on the EasyRA. All results were confirmed with an LC/MS method and are summarized in the table below:

	(0–50 ng/mL) Negative	(>50 ng/mL) Positive
EasyRA	**LC/MS**	**LC/MS**
Negative (<50 ng/mL)	40	0
Positive (>50 ng/mL)	0	40

% Agreement Negative	100%
% Agreement Positive	100%

Imprecision (CLSI, EP5-A2)

Qualitative analysis: Precision of cannabinoid samples (Negative, 37.5 ng/mL and Positive, 62.5 ng/mL) was performed qualitatively (mAbs/min) on one Medica EasyRA Analyzer. Each of the precision samples was analyzed 20 times per run. The mean, standard deviation and the %CV were calculated for within run precision.

Within-Run Imprecision (EP5-A2)
Qualitative Results (Cutoff 50 ng/mL)
Qualitative Results (n=20)

Samples (ng/mL)	Mean (mAbs/Min)	SD (mAbs/Min)	%CV
37.5	212.4	2.0	0.9%
62.5	226.0	2.2	1.0%

% Agreement of Qualitative Precision Results with Target Values

Sample	Acceptance Criteria	Results
37.5 ng/mL	100%	Negative
62.5 ng/mL	100%	Positive

Qualitative analysis: Two samples of cannabinoid were prepared in human urine and analyzed twice a day for 20 days. The samples were tested in qualitative mode and the absorbance change versus time was also measured for each reading. The study followed the protocol defined in EP5-A2. Typical results are as follows:

Total Imprecision (EP5-A2)
Qualitative Results (Cutoff 50 ng/mL)
Qualitative Results (n=40)

Mean (ng/mL)	SD	%CV
36.8	4.1	11.1%
62.7	4.6	7.3%

% Agreement of Qualitative Precision Results with Target Values

Sample	Acceptance Criteria	Results
37.5 ng/mL	100%	Negative
62.5 ng/mL	100%	Positive

Manufactured for:

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