DRI™ Hydrocodone Assay

thermo scientific

IVD **For In Vitro Diagnostic Use Only**

Rx Only

REF 10018053 (500 mL Kit)

10018054 (3 x 18 mL Kit)

Intended Use

The DRI™ Hydrocodone Assay is intended for the qualitative and semi-quantitative detection and estimation of Hydrocodone and its metabolites in human urine at a cutoff of 300 ng/mL. The semi-quantitative mode is for purposes of enabling laboratories to determine an appropriate dilution of specimen for confirmation by a confirmatory method such as LC-MS/MS or GC-MS and permitting laboratories to establish quality control measures.

This assay provides 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] 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

Hydrocodone is a semi-synthetic opioid derived from codeine and thebaine. It is used to relieve moderate to severe pain and to treat a cough. Hydrocodone is prescribed predominantly in the United States. The International Narcotics Control Board reported 99% of the worldwide supply in 2007 was consumed in the United States[2]. Commercial Hydrocodone preparations are often combined with another medication to increase efficacy and reduce adverse effects. It is available as combination drugs with Acetaminophen such as Vicodin and Lortab, Ibuprofen (Vicoprofen) and antihistamines (Hyconine)[1, 3]. In recent years, an increase in the abuse of the prescription pain management drugs Hydrocodone and Hydromorphone has been observed. Analgesic action of Hydrocodone begins 20–30 minutes after taking it and lasts 4–8 hours[4]. Hydrocodone is rapidly metabolized to Hydromorphone and its glucuronide, and can be found in human urine for up to 2-3 days [5-7].

The DRI Hydrocodone Assay is supplied as a liquid ready-to-use homogeneous enzyme immunoassay. The assay uses specific antibody that can detect Hydrocodone and its metabolites. The assay is based on competition between a drug labeled with glucose-6-phosphate dehydrogenase (G6PDH), and free drug from the urine sample, for a fixed amount of specific antibody binding sites. In the absence of free drug from the sample, the specific antibody binds the drug labeled with G6PDH and causes a decrease in enzyme activity. In the presence of free drug, the free drug occupies the antibody binding sites, allowing the drug bound G6PDH to interact with the substrate, resulting in enzyme activity. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is determined spectrophotometrically at 340 nm by measuring the conversion of nicotinamide adenine dinucleotide (NAD) to NADH.

Reagents

REAGENT ***Antibody/Substrate Reagent (A):***

Contains mouse monoclonal anti-Hydrocodone antibody, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.

REAGENT ***Enzyme Conjugate Reagent (E):***

Contains Hydrocodone derivative labeled with glucose-6-phosphate dehydrogenase (G6PDH) in Tris buffer with sodium azide as a preservative.

Additional Materials Required (sold separately):

REF	Kit Description
1664	DRI Negative Calibrator (1 x 10 mL)
1388	DRI Negative Calibrator (1 x 25 mL)
10018079	DRI Hydrocodone Calibrator 100 (1 x 10 mL)
10018080	DRI Hydrocodone Calibrator 300 (1 x 10 mL)
10018081	DRI Hydrocodone Calibrator 500 (1 x 10 mL)
10018082	DRI Hydrocodone Calibrator 1000 (1 x 10 mL)
10018149	DRI Hydrocodone Control Kit (2 x 10 mL)
10026302	DRI Hydromorphone Control (1 x 25 mL)*

**Supports NLCP guidelines for SAMHSA Labs*

⚠ Precautions and Warning

DANGER:

1. The reagents contain ≤0.2% bovine serum albumin (BSA) and ≤0.5% Drug-specific antibody (Mouse). Avoid contact with skin and mucous membranes. Avoid inhalation. May cause skin or inhaled allergic reaction.
2. In the case of accidental spill, clean and dispose of material according to your laboratory's Standard Operating Procedure, local, and state regulations.
3. In the case of damaged packaging on arrival, contact technical support representative.
4. Reagents used in the assay components contain ≤0.09% sodium azide. Avoid contact with skin and mucous membranes. Refer to Safety Data Sheet (SDS) for additional precautions, handling instructions, and accidental exposure treatment.

H317 - May cause allergic skin reaction.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.

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.

Reagent Preparation and Storage

The reagents are ready-to-use; no additional preparation is required. The reagents should be stored refrigerated at 2-8° C. All assay components, opened or unopened, are stable until the expiration date indicated on their respective labels. Do not use reagents beyond the expiration dates.

Specimen Collection and Handling

Collect urine specimens in plastic or glass containers.

Specimens kept at room temperature that do not receive initial test within 7 days[8] of arrival at the laboratory may be placed into a secure refrigeration unit at 2 to 8°C for up to two months.[9] For longer storage prior to analysis or for sample retention after analysis, urine specimens may be stored at -20°C.[9, 10]

Laboratories following the SAMHSA mandatory guidelines should refer to SAMHSA "Short-Term Refrigerated Storage" and "Long-Term Storage" requirements.[11]

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.

Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymatic rates at 340 nm and timing the reaction accurately can be used to perform this immunoassay. Refer to specific application instructions for each analyzer for chemistry parameters before performing the assay.

Assay Procedure

Qualitative analysis

For qualitative analysis, use the DRI Hydrocodone 300 ng/mL calibrator as a cutoff level. This is used as a cutoff reference for distinguishing "positive" from "negative" samples.

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 300 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 urine and retested.

Limitations

1. A positive result from this assay indicates only the presence of Hydrocodone or other cross-reacting substances, 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.

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 Hydrocodone into drug free urine at the cutoff (100%), 25%, 50% & 75% above and below the cutoff and tested in both qualitative and semi-quantitative modes using a Clinical Laboratory and Standards Institute (CLSI) protocol. Results presented below were generated by testing all samples in replicates of 2, twice per day for 20 days, total n=80.

Qualitative Study Analysis

Hydrocodone Spike Concentration (ng/mL)	% of Cutoff 300 ng/mL	LC-MS/MS (ng/mL)	Total Precision (n=80)	
			Number of Determinations	Immunoassay Results
0	-100%	0	80	80 Neg
75	-75%	87	80	80 Neg
150	-50%	170	80	80 Neg
225	-25%	255	80	80 Neg
300	100%	345	80	46 Neg / 34 Pos
375	+25%	442	80	80 Pos
450	+50%	535	80	80 Pos
525	+75%	561	80	80 Pos
600	+100%	664	80	80 Pos

Semi-Quantitative Study Analysis

Hydrocodone Spike Concentration (ng/mL)	% of Cutoff	LC-MS/MS (ng/mL)	Total Precision (n=80)	
			Number of Determinations	Immunoassay Results
0	-100%	0	80	80 Neg
75	-75%	87	80	80 Neg
150	-50%	170	80	80 Neg
225	-25%	255	80	80 Neg
300	100%	345	80	40 Neg / 40 Pos
375	+25%	442	80	80 Pos
450	+50%	535	80	80 Pos
525	+75%	561	80	80 Pos
600	+100%	664	80	80 Pos

Accuracy

One hundred patient samples were analyzed by the DRI Hydrocodone Assay in both qualitative and semi-quantitative modes and the results were compared to LC-MS/MS. The overall concordance between LC-MS/MS and DRI Hydrocodone Assay was 93%.

Qualitative Accuracy study with LC-MS/MS as reference method

Candidate Device Results	Negative	<50% of cutoff concentration by LC/MS (< 150 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration by LC-MS/MS) (150-299 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration by LC-MS/MS) (300-450 ng/mL)	High Positive (Greater than 50% above the cutoff concentration by LC-MS/MS) (>450 ng/mL)
Positive	0	1*	5**	10	39
Negative	31	6	7	1	0

* and ** one sample in each bin has oxycodone concentrations greater than 37,000 ng/mL.

Semi-Quantitative Accuracy study with LC-MS/MS as reference method

Candidate Device Results	Negative	<50% of cutoff concentration by LC/MS (< 150 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration by LC-MS/MS) (150-299 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration by LC-MS/MS) (300-450 ng/mL)	High Positive (Greater than 50% above the cutoff concentration by LC-MS/MS) (>450 ng/mL)
Positive	0	1*	5**	10	39
Negative	31	6	7	1	0

* and ** one sample in each bin has oxycodone concentrations greater than 37,000 ng/mL.

Discordant Result Table for the Discrepant Samples near cutoff

Sample #	Qualitative EIA	LC-MS/MS (ng/mL)				Semi-Quantitative EIA (ng/mL)
		Hydrocodone	Hydromorphone	Hydromorphone 3β-D glucuronide	Adjusted Total #	
33	Positive	143.3	<LLOQ•	67.6	210.9	369
70*	Positive	138.4	<LLOQ	<LLOQ	138	1280
75**	Positive	216.7	<LLOQ	<LLOQ	217	974
76	Positive	198.8	<LLOQ	42.6	241	319
83	Positive	78.4	<LLOQ	110.1	188.5	392
89	Positive	192.3	<LLOQ	56.2	248.5	406
96	Negative	303.3	<LLOQ	50.1	353.4	286

adjusted total LC-MS/MS (ng/mL) Hydrocodone + Hydromorphone + Hydromorphone 3β-D glucuronide.
* and ** Oxycodone positive samples >37,000 ng/mL
• LLOQ = Lowest Limit of Quantitation is 40 ng/mL

Analytical Recovery and Linearity

To demonstrate linearity for purposes of sample dilution and quality control of the entire assay range, a drug free urine was spiked with hydrocodone across the range of calibration curve. Each sample was run in replicates of 5 in semi-quantitative mode and the average was used to determine percent recovery compared to the expected target value. When comparing the result observed (y) and target (x) value, using the least squares regression technique, the regression equation and correlation was as follows y=1.0341-1.9933 and r^2 value was 0.9965.

Target Hydrocodone Value (ng/mL)	Observed Value (ng/mL) n=5	Recovery (%)
0	N/A	N/A
50	47	94
75	76	101
100	108	108
150	171	114
225	250	111
300	302	101
375	398	106
450	472	105
500	527	105
750	844	113
1000	1014	101

Specificity

The cross-reactivity of Hydrocodone and its metabolites was evaluated by adding known amounts of each metabolite to drug-free negative urine.

Cross reactivity of Hydrocodone and its metabolites

Hydrocodone and its metabolites	Tested Concentration (ng/mL)	Pos/Neg	% Cross-reactivity
Neg urine	0	Pos	0%
Hydrocodone	300	Pos	102%
Hydromorphone	310	Pos	97%
Hydromorphone-3β-glucuronide	250	Pos	122%
Norhydrocodone	10,000	Pos	3.1%
Dihydrocodeine	11,000	Pos	2.7%

Cross reactivity of structurally related or unrelated opiate compounds

Structurally related compounds and other opiates	Tested Concentration (ng/mL)	Pos/Neg	% Cross-reactivity
6-Acetyl Morphine	100,000	Neg	< 0.3%
Buprenorphine	100,000	Neg	< 0.3%
Buprenorphine-3β-D-glucuronide	100,000	Neg	< 0.3%
Codeine	150,000	Neg	< 0.2%
Dextromethorphan	250,000	Neg	< 0.2%
EDDP	150,000	Neg	< 0.2%
Fentanyl	100,000	Neg	< 0.3%
Heroin	100,000	Neg	< 0.3%
Levorphanol	18,000	Pos	1.7%
Methadone	100,000	Neg	< 0.3%
Meperidine	100,000	Neg	< 0.3%
Morphine	150,000	Neg	< 0.2%
Morphine-3β-D-glucuronide	70,000	Neg	< 0.4%
Morphine-6β-D-glucuronide	75,000	Neg	< 0.4%
Nalbuphine	150,000	Neg	< 0.3%
Naloxone	15,000	Pos	2.0%
Naltrexone	100,000	Neg	< 0.3%
Norbuprenorphine	100,000	Neg	< 0.3%
Norcodeine	150,000	Neg	< 0.2%
Normorphine	150,000	Neg	< 0.2%
NorOxycodone	100,000	Pos	0.3%
Oxycodone	12,000	Pos	2.5%
Oxymorphone-6β-D-glucuronide	14,000	Pos	2.2%
Oxymorphone	12,000	Pos	2.5%
Tapentadol	100,000	Neg	< 0.3%
Thebaine	100,000	Neg	< 0.3%
Tramadol	100,000	Neg	< 0.3%

The potential cross-reactivity posed by drugs commonly co-administered with Hydrocodone was evaluated by adding each substance to Hydrocodone spiked at low and high controls or drug free urine, at the concentrations indicated. A drug was considered to cross-react if the observed Hydrocodone concentrations result exceeded 300 ng/mL. As shown in the tables below, all the pharmacologic compounds evaluated, including a number of opiate compounds, exhibited minimal cross-reactivity at the concentrations tested.

Structurally unrelated compounds spiked at the concentration listed below into Low and High controls.

Cross Reactants	Spiked Concentration (ng/mL)	Spiked Hydrocodone Concentration	
		Low Control	High Control
Acetaminophen	500,000	Neg	Pos
Acetylsalicylic acid	500,000	Neg	Pos
Amitryptyline	100,000	Neg	Pos
Amoxicillin	100,000	Neg	Pos
Amphetamine	1,000,000	Neg	Pos
Benzoylecgonine	1,000,000	Neg	Pos
Caffeine	100,000	Neg	Pos
Carbamazepine	500,000	Neg	Pos
Chlorpromazine	100,000	Neg	Pos
Clomipramine	10,000	Neg	Pos
Cimetidine	500,000	Neg	Pos
Desipramine	100,000	Neg	Pos
Diphenhydramine	100,000	Neg	Pos
Doxepine	100,000	Neg	Pos
Ephedrine	1,000,000	Neg	Pos
Fluoxethine	100,000	Neg	Pos
Fluphenazine	100,000	Neg	Pos
Ibuprofen	500,000	Neg	Pos
Imipramine	100,000	Neg	Pos
Maprotiline	100,000	Neg	Pos
Nortryptiline	100,000	Neg	Pos
Oxazepam	250,000	Neg	Pos
Phencyclidine	100,000	Neg	Pos
Phenobarbital	100,000	Neg	Pos
Ranitidine	500,000	Neg	Pos
Secobarbital	100,000	Neg	Pos
Thioridazine	100,000	Neg	Pos

Interference

The potential interference of pH and endogenous physiologic substances on recovery of Hydrocodone using DRI Hydrocodone Assay was assessed by spiking known compounds of potentially interfering substances into the low (225 ng/mL) and high (375 ng/mL) controls for 300 ng/mL cutoff. In the presence of the compounds listed below, the controls were detected accurately indicating that these compounds did not show interference in the assay.

Compound	Conc. Tested (mg/dL)	Spiked Hydrocodone Concentration	
		Low Control	High Control
Acetaminophen	10	Neg	Pos
Acetone	500	Neg	Pos
Acetyl Salycylic Acid	10	Neg	Pos
Ascorbic Acid	150	Neg	Pos
Caffeine	10	Neg	Pos
Creatinine	400	Neg	Pos
Ethanol	10	Neg	Pos
Galactose	5	Neg	Pos
Glucose	1000	Neg	Pos
Hemoglobin	150	Neg	Pos
Human Serum Albumin	200	Neg	Pos
Ibuprophen	10	Neg	Pos
Oxalic acid	50	Neg	Pos
Riboflavin	3	Neg	Pos
Sodium Chloride	1000	Neg	Pos
Urea	1000	Neg	Pos
pH	4	Neg	Pos
pH	5	Neg	Pos
pH	6	Neg	Pos
pH	7	Neg	Pos
pH	8	Neg	Pos
pH	9	Neg	Pos
pH	10	Neg	Pos

Specific Gravity

Drug Free urine samples with specific gravity ranging in value from 1.000 to 1.036 g/mL were split and either left unspiked or spiked to a final concentration of either 225 ng/mL or 375 ng/mL (the low and high control concentrations, respectively). These samples were then evaluated in qualitative and semi-quantitative modes. No Interference was observed.

Specific Gravity: 300 ng/mL Cutoff

Spiked Hydrocodone Concentration		
Specific Gravity (g/mL)	Low Control	High Control
1.000	Neg	Pos
1.006	Neg	Pos
1.007	Neg	Pos
1.010	Neg	Pos
1.013	Neg	Pos
1.018	Neg	Pos
1.021	Neg	Pos
1.025	Neg	Pos
1.028	Neg	Pos
1.034	Neg	Pos
1.036	Neg	Pos

References

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Glossary:

http://www.thermofisher.com/symbols-glossary

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