DRI® Oxycodone Assay

thermo scientific

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 10015632 (3 x 18 mL Kit)
100248 (70 mL Kit)
100249 (500 mL Kit)

Intended Use
The DRI® Oxycodone Assay is intended for the qualitative and semi-quantitative determination of oxycodone in human urine at cutoffs of 100 and 300 ng/mL. The assay provides a simple and rapid analytical screening procedure to detect oxycodone in human urine.

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

Summary and Explanation of the Test
Oxycodone is a semi-synthetic opioid prescribed for pain management in patients with moderate to severe pain. It is similar to codeine and morphine in its analgesic properties but it is more potent than morphine and has higher dependence potential. The drug oxycodone is supplied as OxyContin® (Oxycodone HCl) or in combination with aspirin (Percodan®) or acetaminophen (Percocet®).[1] Drug abusers crush the pills into powder and snort them for faster effect which may result in a potentially fatal outcome. According to Drug Abuse Warning Network (DAWN), there has been a dramatic increase in oxycodone related deaths.[2,3] Oxymorphone, noroxycodone and noroxymorphone are the only known metabolites of oxycodone.[2] The metabolite, oxymorphone, is a potent narcotic analgesic, while the other two metabolites are relatively inactive. From 33-61% of a single dose of oxycodone is excreted in urine within 24 hours as unconjugated oxycodone (13-19%), conjugated oxycodone (7-29%), and conjugated oxymorphone (13-14%).[4]

The DRI Oxycodone Assay is supplied as a liquid ready-to-use homogeneous enzyme immunoassay. The assay uses specific antibodies that can detect oxycodone and oxymorphone without any significant cross-reactivity to other opiate compounds. 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. 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
Antibody/Substrate Reagent:
Contains mouse monoclonal anti-oxycodone derivative antibody, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.
Enzyme Conjugate Reagent:
Contains oxycodone 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, 10 mL
1388	DRI Negative Calibrator, 25 mL
100250	DRI Oxycodone Calibrator 100, 10 mL
100251	DRI Oxycodone Calibrator 300, 10 mL
100252	DRI Oxycodone Calibrator 500, 10 mL
100253	DRI Oxycodone Calibrator 1000, 10 mL
DOAT-2	MAS® DOA Total – Level 2
DOAT-3	MAS® DOA Total – Level 3
DOAT-4	MAS® DOA Total – Level 4
DOAT-5	MAS® DOA Total – Level 5

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

DANGER: DRI Oxycodone Assay contains ≤0.2% bovine serum albumin (BSA) and ≤0.5% Drug-specific antibody (Mouse).
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.

Reagents used in the assay components contain ≤0.09% 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.

Do not use the reagents beyond their expiration dates.

Reagent Preparation and Storage
The reagents are ready-to-use. No additional reagent preparation is required. The reagents should be stored refrigerated (2-8°C). All assay components, opened or unopened, are stable until the expiration date indicated on their respective labels. Do not use the reagents beyond their expiration dates.

Specimen Collection and Handling
Collect urine specimens in plastic or glass containers. Testing of fresh urine specimens is suggested.

Specimens kept at room temperature that do not receive initial test within 7 days[5] of arrival at the laboratory may be placed into a secure refrigeration unit at 2 to 8°C for up to two months.[6] For longer storage prior to analysis or for sample retention after analysis, urine specimens may be stored at -20°C.[6, 7]

Laboratories following the SAMHSA mandatory guidelines should refer to SAMHSA "Short-Term Refrigerated Storage" and "Long-Term Storage" requirements.[8]

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
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 assay.

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

Quality Control and Calibration
Qualitative analysis
For qualitative analysis of samples, use either the Oxycodone 100 Calibrator, or the Oxycodone 300 calibrator, as a cutoff level.

Semi-quantitative analysis
For semiquantitative analysis, use all calibrators.

Good laboratory practice suggests the use of control specimens to ensure proper assay performance. Use controls near the cutoff calibrator to validate the calibration. Control results must fall within established ranges, as determined by laboratory procedures and guidelines. If 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.

Calibration Frequency
Recalibration is recommended
- After calibrator or reagent lot change
- After instrument maintenance is performed
- As required following quality control procedures

See below for calibration frequency recommendations for Hitachi analyzers. For other analyzers, refer to the instrument-specific application sheet.

NOTE: Reassess control targets and ranges following a change of reagent lot.

Results and Expected Values

Qualitative

Either the 100 or the 300 calibrator can be used as a cutoff reference for distinguishing "positive" from "negative" samples. 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.

Semi-quantitative

A rough estimate of drug concentration in the samples can be obtained by running a standard curve with all calibrators and quantitating samples 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 only the presence of oxycodone or oxymorphone and does not necessarily correlate with the extent of physiological and psychological effects.
2. Performance characteristics for the DRI Oxycodone Assay have not been established with body fluids other than human urine.
3. This DRI Oxycodone Assay was validated on analyzers utilizing an integral cell wash. If your analyzer does not have an integral cell wash, contact your local Microgenics representative.
4. 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.
5. It is possible that substances other than those investigated in the specificity study may interfere with the test and cause false results.

Typical Performance Characteristics

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

Precision

The DRI Oxycodone Controls (75, 125, 225, and 375 ng/mL) and cutoff calibrators (100 and 300 ng/mL) were tested in qualitative (mA) and semi-quantitative (ng/mL) mode using a modified NCCLS protocol. Results presented below were generated by testing all samples in replicates of 6, twice per day for 10 days using packaged reagents and controls on the Hitachi 717.

Qualitative (mA/min)

Calibrator/Control	100 ng/mL Cutoff					
n=120	Within-run Precision			Total Precision		
	Mean	SD	% CV	Mean	SD	% CV
75 ng/mL	348	2.1	0.6	348	2.9	0.8
100 ng/mL	371	1.9	0.5	371	3.2	0.9
125 ng/mL	389	2.0	0.5	389	3.1	0.8

Qualitative (mA/min)

Calibrator/Control	300 ng/mL Cutoff					
n=120	Within-run Precision			Total Precision		
	Mean	SD	% CV	Mean	SD	% CV
225 ng/mL	429	2.2	0.5	429	3.7	0.9
300 ng/mL	458	2.4	0.5	458	4.1	0.9
375 ng/mL	479	2.4	0.5	479	3.8	0.8

Semi-quantitative (ng/mL)

Calibrator/Control						
n=120	Within-run Precision			Total Precision		
	Mean	SD	% CV	Mean	SD	% CV
75 ng/mL	73	2.4	3.3	73	2.9	4.0
100 ng/mL	98	2.9	2.9	98	3.6	3.7
125 ng/mL	123	2.4	2.0	123	4.9	4.0
225 ng/mL	227	5.0	2.2	227	8.2	3.6
300 ng/mL	303	9.0	3.0	303	11.5	3.8
375 ng/mL	375	10.2	2.7	375	14.7	3.9

Linearity

A urine pool containing a known high concentration of oxycodone was serially diluted with a human urine pool free of oxycodone to produce samples with oxycodone concentrations between 50 and 100 ng/mL. The oxycodone concentration for each dilution was determined using the DRI Oxycodone Assay. The percent recovery was calculated as the quotient of the observed to expected value. Results demonstrated that the observed oxycodone concentration for serially diluted specimens were within ±10% of expected values indicating the linearity of the assay with successively diluted specimens originating from a single high pool.

Cutoff Characterization

Oxycodone samples around the cutoff were prepared by the addition of oxycodone stock solution to negative urine. The samples were targeted at the following control concentrations, 75 ng/mL and 125 ng/mL (± 25% of the 100 ng/mL cutoff) and 225 ng/mL and 375 ng/mL (± 25% of the 300 ng/mL cutoff). The samples were assayed in replicates of 21. Cutoff characterization was deemed acceptable if the observed oxycodone concentration for 95% of the 21 replicates was appropriately greater or lesser than the cutoff calibrator concentration. For all 21 replicates, the 75 ng/mL and 225 ng/mL samples assayed correctly, as less than their respective cutoff calibrators 100 % of the time. The 125 ng/mL and 375 ng/mL samples assayed as greater than their respective cutoff calibrators 100 % of the time.

Sensitivity

The sensitivity of the assay using the negative calibrator is 4.9 ng/mL.

Accuracy

One hundred and forty-four samples were analyzed by the DRI Oxycodone Assay in both the qualitative and semi-quantitative modes and the results were compared to the RapidOne™ OxyTest and to GC/MS. As the RapidOne™ OxyTest is a qualitative method for the detection of oxycodone at 100 ng/mL, only qualitative results at the 100 ng/mL cutoff for the DRI Oxycodone Assay were compared.

One hundred and forty-four urine samples were assayed with the DRI Oxycodone Assay at 100 and 300 ng/mL cutoff on the Thermo Scientific Indiko and the Hitachi 717 analyzers.

Qualitative

The overall concordance between the DRI Oxycodone Assay and the RapidOne™ Oxy Test was 91.7%. The 12 samples detected as positive by the RapidOne™ Oxy Test and negative by the DRI Oxycodone Assay were confirmed by GC/MS to have oxycodone concentrations ≤100 ng/mL. At the 100 ng/mL cutoff, the overall concordance between DRI Oxycodone Assay and the GC/MS was 97.2%. At the 300 ng/mL cutoff, the overall concordance between DRI Oxycodone Assay and GC/MS was also 97.2%.

100 ng/mL Cutoff

RapidOne™ Oxy

DRI	+	-
+	65	0
-	12	67

100 ng/mL Cutoff

GC/MS

DRI	+	-
+	61	4†
-	0	79

300 ng/mL Cutoff

GC/MS

DRI	+	-
+	39	1‡
-	3	101

† Oxycodone concentrations ranged from 55-81 ng/mL.
‡ Sample rate is 24 units above the cutoff in DRI Oxycodone Assay.

Semi-quantitative

The same 144 samples were assayed in tandem using GC/MS and the semi-quantitative mode of the DRI Oxycodone Assay. At the 100 ng/mL cutoff, the overall concordance between the DRI Oxycodone Assay and GC/MS was also 99.3% (143/144). One sample was positive by the DRI Oxycodone Assay and negative by GC/MS. At the 300 ng/mL cutoff, the overall concordance between the DRI Oxycodone Assay and GC/MS was 97.2% (140/144). One sample was positive and three samples were borderline negative by the DRI Oxycodone Assay.

100 ng/mL Cutoff (Semi-quantitative)

GC/MS

DRI	+	-
+	61	1*
-	0	82

300 ng/mL Cutoff (Semi-quantitative)

GC/MS

DRI	+	-
+	40	1
-	3	100

*Oxycodone concentration was 55 ng/mL by GC/MS with a DRI value of 103 ng/mL.

Specificity

The cross-reactivity of oxycodone metabolites, oxymorphone, noroxymorphone and noroxycodone, was evaluated by adding known amounts of each metabolite to oxycodone free urine. A metabolite was determined to cross react with oxycodone if recovery observed for the metabolite spiked specimen was greater than 1% of the estimated target concentration. As indicated by the results in the table below, oxymorphone exhibits 103% cross reactivity with oxycodone; noroxymorphone and noroxycodone show no evidence of significant cross-reactivity.

Compound	Concentration tested (ng/mL)	Recovery (ng/mL)	% Cross-reactivity
Oxycodone	300	300	100
Oxymorphone	300	308	103
Noroxymorphone	500,000	303.5	<0.1
Noroxycodone	50,000	41.5	<0.1

The potential cross-reactivity posed by drugs commonly coadministered with oxycodone was evaluated by adding each substance to oxycodone free urine at the concentration indicated. A drug was considered to cross-react if the observed oxycodone concentration exceeded 100 ng/mL, the lowest cutoff for the DRI Oxycodone Assay. As shown in the tables below, all of the pharmacologic compounds evaluated, including a number of the opiate compounds, exhibited no cross-reactivity at the concentrations listed. Please note that some substances including 6-acetyl morphine, codeine, dihydrocodeine, heroin, hydrocodone, hydromorphone, levorphanol, naloxone, naltrexone gave results between 75 and 99 ng/mL i.e., within 25% of the cutoff of 100 ng/mL.

Structurally related opiate compounds that tested negative at 100 ng/mL cutoff.

Compound	Concentrations (µg/mL)
6-Acetyl Morphine	75
Codeine	500
Dihydrocodeine	200
Heroin	300
Hydrocodone	200
Hydromorphone	40
Levorphanol	200
Morphine	350
Morphine-3-glucuronide	950
Naloxone	300
Norcodeine	1,000
Normorphine	1,000

Structurally unrelated compounds that tested negative at 100 ng/mL cutoff.

Compound	Concentrations (µg/mL)
Acetaminophen	1,000
Acetylsalicylic acid	1,000
Amitriptyline	500
Amoxcillin	500
Amphetamine	2,000
Benzoylecgonine	2,000
Caffeine	1,000
Carbamazepine	1,000
Chlorpromazine	2,000
Clomipramine	1,000
Cimetidine	1,000
Desipramine	1,000
Dextromethorphan	200
Doxepine	200
Ephedrine	2,000
Fentanyl	200
Fluoxethine	1,000
Fluphenazine	500
Ibuprofen	1,000
Imipramine	1,000
Maprotiline	1,000
Meperidine	1,000
Methadone	1,000
Metroniazole	2,000
Nalbuphine	1,000
Nortriptyline	500
Oxazepam	500
Phencyclidine	1,000
Phenobarbital	1,000
Ranitidine	3,000
Secobarbital	1,000
Talwin	500
Thebaine	20
Thioridazine	1,000
Tramadol	500

Interference

The potential interference of pH and endogenous physiologic substances on recovery of oxycodone using the DRI Oxycodone Assay was assessed by spiking known amounts of potentially interfering substances into the low (225 ng/mL) and high (375 ng/mL) controls for the 300 ng/mL cutoff. Oxycodone concentration for each specimen (substance and final concentration noted in the table below) was determined and the percent recovery calculated as the quotient of the spiked to control value. The table below shows the substance and final concentration at which the observed oxycodone concentrations for spiked controls are within ±1% of the expected control dose. No interference was observed by the addition of the compounds upto the concentrations listed below.

Compound	Concentrations (mg/dL)
Acetone	1,000
Ascorbic Acid	1,500
Creatinine	500
Ethanol	1,000
Galactose	10
Glucose	3,000
Hemoglobin	300
Human Serum Albumin	500
Oxalic Acid	100
Riboflavin	7.5
Sodium chloride	1,000
Urea	2,000
pH	3-11

References

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3. Cone E.J., etal, Oxycodone Involvement in Drug Abuse Deaths: A DAWN-Based Classification Scheme applied to an Oxycodone Postmortem Database Containing over 1000 Cases. J. Anal. Toxicol. 2003, 27: 57-67.
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7. C52-A2, Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline – Second Edition, *Clinical and Laboratory Standards Institute (CLSI)* (April 2007)
8. *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
9. Data on file at Microgenics, a part of Thermo Fisher Scientific.

Glossary:

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

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