DRI® Cocaine Metabolite Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use Only**

REF **10014593 (3 x 18 mL)**
0055 (100 mL Kit)
0056 (500 mL Kit)

Intended Use

The DRI Cocaine Metabolite Enzyme Immunoassay is a homogeneous enzyme immunoassay intended for the qualitative and semi-quantitative determination of benzoylecgonine (Cocaine Metabolite) in human urine with either 300 ng/mL or 150 ng/mL as a cutoff calibrator.

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.[1,2] Clinical and professional judgment should be applied to any drug of abuse test result, particularly when preliminary results are used. Tests for cocaine metabolite cannot distinguish between abused drugs and certain prescribed medications.

Summary and Explanation of the Test

Cocaine (benzoylmethylecgonine), is derived from the plant species Erythroxylon coca, which is widely grown in South America.[3-5]

Cocaine is a very common illicit drug and is popularly abused in the US.[3,4,6] Cocaine abuse can produce euphoria, arousal, garrulousness, alertness, anxiety, insomnia, hyperactivity, paranoia, severe psychosis, and even suicide.[3,6,7]

Cocaine is rapidly metabolized, with less than 5% excreted unchanged in the urine.[4,6,7] The two major metabolites, which result from enzymatic and nonenzymatic hydrolysis, are benzoylecgonine and ecgonine methyl ester.[4,6-9] The metabolites may be detectable in urine for up to 3 weeks after long term, heavy use of cocaine.[10,11]

The DRI® Cocaine Metabolite Assay is a homogeneous enzyme immunoassay using ready-to-use liquid reagents.[12] The assay uses a specific antibody, which can detect benzoylecgonine in urine. The assay is based on the competition of an enzyme glucose-6-phosphate dehydrogenase (G6PDH) labeled drug and the drug from the urine sample for a fixed amount of specific antibody binding sites. In the presence of free drug from the sample, the free drug occupies the antibody binding sites, allowing the drug-labeled G6PDH to interact with the substrate, resulting in enzyme activity. In the absence of drug from the sample, the specific antibody binds to the drug labeled with G6PDH and the enzyme activity is inhibited. This phenomenon creates a direct relationship between the drug concentration in the urine and the enzyme activity. The enzyme G6PDH activity is determined spectrophotometrically at 340 nm by measuring its ability to convert nicotinamide adenine dinucleotide (NAD) to NADH.

Reagents

Antibody/Substrate Reagent (R1)
Contains mouse monoclonal anti-benzoylecgonine antibody, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as preservative.

Enzyme Conjugate Reagent (R2)
Contains benzoylecgonine analog labeled with glucose-6-phosphate dehydrogenase (G6PDH) in HEPES buffer with sodium azide as preservative

Additional Materials Required (sold separately)

REF	Kit Description
1664	DRI Negative Calibrator, 10 mL
1388	DRI Negative Calibrator, 25 mL
1588	DRI Multi-Drug Calibrator 1, 10 mL
1589	DRI Multi-Drug Calibrator 1, 25 mL
1591	DRI Multi-Drug Calibrator 2, 10 mL
1592	DRI Multi-Drug Calibrator 2, 25 mL
1594	DRI Multi-Drug Calibrator 3, 10 mL
1595	DRI Multi-Drug Calibrator 3, 25 mL
1597	DRI Multi-Drug Calibrator 4, 10 mL
1598	DRI Multi-Drug Calibrator 4, 25 mL
DOAT-2	MAS® DOA Total – Level 2, 6 x 18 mL
DOAT-3	MAS® DOA Total – Level 3, 6 x 18 mL
DOAT-4	MAS® DOA Total – Level 4, 6 x 18 mL
DOAT-5	MAS® DOA Total – Level 5, 6 x 18 mL

⚠ Precautions and Warnings

1. **DANGER:** This test is for in vitro diagnostic use only. The reagents are harmful if swallowed.
2. The DRI Cocaine Metabolite Enzyme Immunoassay contains ≤0.2% bovine serum albumin (BSA) and ≤0.5% Drug-specific antibody (Mouse).
3. 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.
4. Do not use the reagents beyond their expiration dates.

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. 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 the reagents beyond their expiration dates.

Specimen Collection and Handling

Collect urine specimens in plastic or glass containers. Fresh urine specimens should be used. The *Mandatory Guidelines for Federal Workplace Drug Testing Programs* 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 set at 2-8°C.[2]

An effort should be made to keep pipetted samples free of gross debris. Centrifuge highly turbid specimens before analysis. Adulteration 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

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 assay.

Before performing this assay, refer to the analyzer-specific protocol sheet that contains parameters and/or additional instructions for use.

Quality Control and Calibration[13]

Good laboratory practice suggests the use of control specimens to ensure proper assay performance. Use controls near the cutoff calibrator to validate the calibration. It is recommended that two controls be run; one with a concentration 25% above the selected cutoff and the other with a concentration 25% below the selected cutoff. Use MGC Select DAU Control Set for the 150 cutoff quality control and MGC Primary DAU control for the 300 ng/mL quality control. Ensure that control results are within the established ranges determined by laboratory practices and guidelines. If control results fall outside the established ranges, specimen 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 control ranges and calibration frequency.

Qualitative Analysis
For qualitative analysis of samples, use the DRI Multi-Drug Urine Calibrator 1, which contains 150 ng/mL benzoylecgonine, or DRI Multi-Drug Calibrator 2, which contains 300 ng/mL benzoylecgonine as a cutoff level. The cutoff calibrator is used as a reference for distinguishing "positive" from "negative" samples.

Semi-quantitative Analysis
For semi-quantitative analysis of samples, use all calibrators: Negative Calibrator, Multi-Drug Calibrator 1, 2, 3 and 4 to create a standard curve to analyze the results.

Results and Expected Values

Qualitative Analysis

A sample that exhibits a change in absorbance value (ΔA) equal to or greater than the value obtained with the cutoff calibrator is considered a "positive" result. A sample that exhibits a change in absorbance value (ΔA) lower than the value obtained with the cutoff calibrator is considered a "negative" result. Refer to analyzer specific application sheet for additional information.

Semi-quantitative Analysis

A rough estimate of drug concentration in the samples can be obtained by running a standard curve with calibrators and then quantifying samples off that curve. Samples with results above the highest calibrator concentration (1000 ng/mL) should be diluted with negative urine and retested. The semi-quantitation of positive results enables laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as GC/MS. It also permits the laboratory to establish quality control procedures and assess control performance. Refer to the analyzer specific application sheet for detailed information.

Limitations

1. A positive result from this assay indicates only the presence of cocaine metabolite and does not necessarily correlate with the extent of physiological and psychological effects.
2. A positive result by this assay should be confirmed by another nonimmunological method such as GC or GC/MS.
3. The test is designed for use with human urine only.
4. It is possible that other substances and/or factors (technical or procedural) other than those investigated in the specificity study may interfere with the test and cause false results.

Specific Performance Characteristics

Typical performance results obtained on the Beckman Coulter AU 680 and the Thermo Scientific Indiko clinical chemistry analyzers are shown below[14]. The results obtained in each laboratory may differ from these data.

Sensitivity

Analytical Sensitivity (limit of blank): defined as the lowest concentration that can be differentiated from the negative urine calibrator with 95% confidence, is 4.44 ng/mL.

Functional Sensitivity (limit of quantitation): defined as the lowest concentration that results in a CV=20% with upper 95% confidence interval, is 8.5 ng/mL.

Precision

A benzoylecgonine solution (1 mg/mL) was added to each of four samples obtained from a human urine sample pool to achieve concentrations that span the assay range. The samples were tested for precision in qualitative and semi-quantitative modes. Following a CLSI (EP05-A2) precision protocol, samples were tested in 2 replicates per run, 2 runs per day for 20 days, total N=80.

AU680 Qualitative

Concentration of sample, ng/mL	Number of determinations	150 Cutoff # Neg / # Pos	300 Cutoff # Neg / # Pos
0	80	80 / 0	80 / 0
75	80	80 / 0	80 / 0
112.5	80	80 / 0	80 / 0
187.5	80	0 / 80	80 / 0
300	80	0 / 80	0 / 80
375	80	0 / 80	0 / 80
500	80	0 / 80	0 / 80

AU680 Semi-quantitative

Concentration of sample, ng/mL	Result Mean mg/mL	150 Cutoff # Neg / # Pos	300 Cutoff # Neg / # Pos	Within-run Precision		Total Precision	
				SD ng/mL	% CV	SD ng/mL	% CV
75	80.1	80 / 0	80 / 0	2.1	2.7	2.3	2.9
112.5	115.4	80 / 0	80 / 0	2.0	1.8	2.5	2.2
187.5	202.3	0 / 80	80 / 0	3.8	1.9	4.8	2.4
225	250.9	0 / 80	80 / 0	4.0	1.6	4.4	1.8
375	403.1	0 / 80	0 / 80	8.4	2.1	10.0	2.5
500	502.7	0 / 80	0 / 80	14.8	2.9	19.1	3.8

Cutoff Characterization

Spiked samples at the concentrations same as the cutoff calibrators and ± 25% controls were prepared by spiking benzoylecgonine into negative urine. The samples were assayed in replicates of 21 in both qualitative and semi-quantitative modes. Results show that in qualitative mode, the control levels were detected accurately with mean response of the negative control plus 2 SD did not overlap with the mean response of the cutoff calibrator, and the mean response of the positive control minus 2 SD did not overlap with the mean response of the cutoff calibrator. In semi-quantitative mode, the negative controls recovered less than the cutoff calibrators and the positive controls recovered greater than the cutoff calibrators. The precision was less than 5% CV for semi-quantitative mode.

Dilution Recovery and Linearity

A high patient urine sample containing around 1000 ng/mL benzoylecgonine was serially diluted with analyte-free urine in 10% increments and tested by 5 replicates in semi-quantitative mode. All samples were recovered within ± 10% error of the expected value and the r-value was 0.9981.

Interference

The potential effect of endogenous and exogenous urine substances and pH on the recovery of benzoylecgonine using DRI Cocaine Metabolite Assay was assessed by spiking known amounts of potentially interfering substances into the negative and positive levels (± 25% of cutoff) for both cutoffs. The compounds were determined to not interfere with the assay if the rate of each negative sample was below its cutoff rate, and if all samples were recovered within 20% error of their expected concentrations. No interference was observed by the addition of the compounds up to the concentrations listed below.

Qualitative (NEG/POS)

Compound	Cmpd. Conc.	150 cutoff		300 cutoff	
		Neg level	Pos level	Neg level	Pos level
Acetaminophen	100 µg/mL	NEG	POS	NEG	POS
Acetone	1 g/dL	NEG	POS	NEG	POS
Ascorbic acid	1 g/dL	NEG	POS	NEG	POS
Aspirin	100 µg/mL	NEG	POS	NEG	POS
Caffeine	100 µg/mL	NEG	POS	NEG	POS
Creatinine	500 mg/dL	NEG	POS	NEG	POS
Ethanol	1 g/dL	NEG	POS	NEG	POS
Galactose	10 mg/dL	NEG	POS	NEG	POS
γ-globulin	500 mg/dL	NEG	POS	NEG	POS
Glucose	3 g/dL	NEG	POS	NEG	POS
Hemoglobin	150 mg/dL	NEG	POS	NEG	POS
Human serum albumin	500 mg/dL	NEG	POS	NEG	POS
Ibuprofen	100 µg/mL	NEG	POS	NEG	POS
Oxalic Acid	100 mg/dL	NEG	POS	NEG	POS
pH range	3-11	NEG	POS	NEG	POS
Riboflavin	7.5 mg/dL	NEG	POS	NEG	POS
Sodium chloride	1 g/dL	NEG	POS	NEG	POS
Specific gravity range	1.004-1.039 g/mL	NEG	POS	NEG	POS
Urea	1.25 g/dL	NEG	POS	NEG	POS

Semi-quantitative (ng/mL)

Compound	Conc. µg/mL	Compound	Conc. µg/mL
Acetaminophen	1,000	Calcium Carbonate	5000
Acetylsalicylic acid	1,000	Chlorpromazine	500
Acyclovir	100	Chlorzoxazone	1000
Albuterol	1000	Clonidine	100
Amikacin	1000	Codeine	1000
Amitryptyline	100	Dapsone	10
Amobarbital	1000	Dextromethorphan	100
Amoxicillin	1000	Diphenhydramine	1000
Amphetamine	1000	Doxepine	500
Azithromycin	100	Doxycycline Hyclate	100
Benzocaine	1000	Fentanyl	10
Buprenorphine	10	Fluconazole	100
Bupropion	100	Fluoxetine	50
Caffeine	100	Gabapentin	100
Gentamicin	1000	Oxazepam	1000
Hydroxyzine	100	Paroxetine	100
Hyoscyamine HCl	100	Phencyclidine	1000
Ibuprofen	5000	Phenelzine	100
Indomethacin	100	Phenobarbital	1000
Lamotrigine	1000	Promethazine	100
Levofloxacin	100	Propoxyphene	1000
Lidocaine	1000	Ranitidine	100
Lithium heparin	5000	Risperidone	100

Semi-quantitative (ng/mL) con't

Compound	Conc. µg/mL	Compound	Conc. µg/mL
Loratadine	500	Scopolamine	1000
Meperidine	1000	Secobarbital	1000
Mesoridazine	1000	Spironolactone	1000
Methadone	1000	Stavudine	1.0
Methylphenidate	100	Terbinafine	1000
Metoclopramide	1000	Thiopental	1000
Metronidazole	100	Thioridazine	1000
Morphine	200	Tobramycin	1000
Nalbuphine	1000	Tolmetin	1000
Naltrexone	1000	Tramadol	500
Naproxen	5000	Trazodone	1000
Norfluoxetine HCl	1000	Trimethoprim	5000
Ofloxacin	100	Vancomycin	1000
Omeprazole	100	Venlafaxine	1000

Specificity

The cross-reactivity of parent drug, metabolites, and drugs commonly found in specimens was evaluated by adding known amounts of each substance to benzoylecgonine-free urine. A compound producing negative results compared to both the 150 ng/mL and 300 ng/mL cutoff calibrators was considered to have no cross-reactivity.

The following parent compound and metabolites, when tested with DRI Cocaine Metabolite assay, produced a positive result at the concentrations listed below with the exception of ecgonine methyl ester which gave negative result.

Compound	150 ng/mL cutoff	300 ng/mL cutoff
	Concentration, µg/mL	Concentration, µg/mL
Benzoylecgonine	0.150	0.300
Cocaine	25	50
Cocaethylene	30	70
Ecgonine	50	80
Ecgonine Methyl Ester	100	100

Structurally unrelated compounds and/or concurrently used drugs produced a negative result compared to both the 150 ng/mL and 300 ng/mL cutoff calibrators at the concentrations listed below.

Compound	Conc. µg/mL	Compound	Conc. µg/mL
Acetaminophen	1,000	Calcium Carbonate	5000
Acetylsalicylic acid	1,000	Chlorpromazine	500
Acyclovir	100	Chlorzoxazone	1000
Albuterol	1000	Clonidine	100
Amikacin	1000	Codeine	1000
Amitryptyline	100	Dapsone	10
Amobarbital	1000	Dextromethorphan	100
Amoxicillin	1000	Diphenhydramine	1000
Amphetamine	1000	Doxepine	500
Azithromycin	100	Doxycycline Hyclate	100
Benzocaine	1000	Fentanyl	10
Buprenorphine	10	Fluconazole	100
Bupropion	100	Fluoxetine	50
Caffeine	100	Gabapentin	100
Gentamicin	1000	Oxazepam	1000
Hydroxyzine	100	Paroxetine	100
Hyoscyamine HCl	100	Phencyclidine	1000
Ibuprofen	5000	Phenelzine	100
Indomethacin	100	Phenobarbital	1000

Table con't

Compound	Conc. µg/mL	Compound	Conc. µg/mL
Lamotrigine	1000	Promethazine	100
Levofloxacin	100	Propoxyphene	1000
Lidocaine	1000	Ranitidine	100
Lithium heparin	5000	Risperidone	100
Loratadine	500	Scopolamine	1000
Meperidine	1000	Secobarbital	1000
Mesoridazine	1000	Spironolactone	1000
Methadone	1000	Stavudine	1.0
Methylphenidate	100	Terbinafine	1000
Metoclopramide	1000	Thiopental	1000
Metronidazole	100	Thioridazine	1000
Morphine	200	Tobramycin	1000
Nalbuphine	1000	Tolmetin	1000
Naltrexone	1000	Tramadol	500
Naproxen	5000	Trazodone	1000
Norfluoxetine HCl	1000	Trimethoprim	5000
Ofloxacin	100	Vancomycin	1000
Omeprazole	100	Venlafaxine	1000

Accuracy

Eighty eight clinical specimens were tested using the DRI Cocaine Metabolite Assay on the Beckman Coulter AU 680 clinical chemistry analyzer and GC/MS. The results are presented as follows:

Qualitative Stratified Results

DRI	Low Negative by GC/MS (less than -50%)	Near Cutoff Negative by GC/MS (between -50% and cutoff)	Near Cutoff Positive by GC/MS (between cutoff and +50%)	High Positive by GC/MS (greater than +50%)	Percent Agreement with GC/MS
150 ng/mL Cutoff					
Positive	0	1	5	43	100%
Negative	35	4	0	0	98%
300 ng/mL Cutoff					
Positive	0	2	15	24	98%
Negative	40	6	1	0	96%

GC/MS Summary of Discordant Qualitative Results

Cutoff Value (ng/mL)	DRI Result	GC/MS (ng/mL)	Major Drug Present by GC/MS
100	POS	88	Benzoylecgonine
300	POS	299	Benzoylecgonine
300	POS	279	Benzoylecgonine
300	NEG	375	Benzoylecgonine

Semi-quantitative Stratified Results

DRI	Low Negative by GC/MS (less than -50%)	Near Cutoff Negative by GC/MS (between -50% and cutoff)	Near Cutoff Positive by GC/MS (between cutoff and +50%)	High Positive by GC/MS (greater than +50%)	Percent Agreement with GC/MS
150 ng/mL Cutoff					
Positive	0	1	5	43	100%
Negative	35	4	0	0	98%
300 ng/mL Cutoff					
Positive	0	2	14	24	95%
Negative	40	6	2	0	96%

GC/MS Summary of Discordant Semi-quantitative Result

Cutoff Value (ng/mL)	DRI Result (ng/mL)	GC/MS (ng/mL)	Major Drug Present by GC/MS
100	162 (POS)	88	Benzoylecgonine
300	294 (NEG)	399	Benzoylecgonine
300	329 (POS)	299	Benzoylecgonine
300	320 (POS)	279	Benzoylecgonine
300	253 (NEG)	375	Benzoylecgonine

Qualitative Analysis: The overall concordance between the DRI Cocaine Metabolite Assay on the Beckman Coulter AU680 and the GC/MS was 99% for the 150 cutoff and 97% for the 300 cutoff.

Qualitative

		150 ng/mL GC/MS	
		+	-
DRI	+	48	1[a]
	-	0	39

		300 ng/mL GC/MS	
		+	-
DRI	+	39	2[b]
	-	1[c]	46

[a] GC/MS results showed this sample was negative at 88 ng/mL.

[b] They were borderline positive by DRI and borderline negative by GC/MS (299 ng/mL and 279 ng/mL).

[c] DRI method gave near cutoff negative at 253 ng/mL. GC/MS showed it's near cutoff positive at 375 ng/mL.

Semi-quantitative Analysis: The overall concordance between the DRI Cocaine Metabolite Assay on the Beckman Coulter AU680 and the GC/MS was 99% for the 150 cutoff and 95% for the 300 cutoff.

Semi-quantitative

		150 ng/mL GC/MS	
		+	-
DRI	+	48	1[a]
	-	0	39

		300 ng/mL GC/MS	
		+	-
DRI	+	38	2[c]
	-	2[b]	46

[a] The same sample listed in Qualitative Analysis above (1[a]).

[b] The same sample listed in Qualitative Analysis above (2[c]).

[c] GC/MS showed they were near cutoff positive at 399 and 375 ng/mL DRI method gave near cutoff negative at 294 and 253 ng/mL.

References

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13. Data on traceability are on file at Microgenics, a part of Thermo Fisher Scientific.
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