DRI® Cocaine Metabolite Assay

microgenics

For In Vitro Diagnostic Use

Catalog No.: 0055 (100 mL Kit)
0056 (500 mL Kit)

Intended Use

The DRI® Cocaine Metabolite assay is intended for the qualitative and semiquantitative determination of benzoylecgonine (cocaine metabolite) 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.[1,2] 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

Cocaine is a very common illicit drug. When ingested, it is rapidly metabolized and excreted into urine as benzoylecgonine (the major metabolite of cocaine) within four hours. Detection of benzoylecgonine in urine indicates use of cocaine.

The DRI Cocaine Metabolite Assay is a homogeneous enzyme immunoassay using ready-to-use liquid reagents.[3] 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 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. 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. Contains benzoylecgonine analog labeled with glucose-6-phosphate dehydrogenase (G6PDH) in HEPES buffer with sodium azide as preservative.

Additional Materials Required (sold separately):

Catalog No.
1664 DRI Negative Calibrator, 10 mL
1388 DRI Negative Calibrator, 25 mL
1588 DRI MultiDrug Calibrator 1, 10 mL
1589 DRI MultiDrug Calibrator 1, 25 mL
1591 DRI MultiDrug Calibrator 2, 10 mL
1592 DRI MultiDrug Calibrator 2, 25 mL
1594 DRI MultiDrug Calibrator 3, 10 mL
1595 DRI MultiDrug Calibrator 3, 25 mL
1597 DRI MultiDrug Calibrator 4, 10 mL
1598 DRI MultiDrug Calibrator 4, 25 mL
1599 DRI MultiDrug Urine Control 1, 10 mL
1553 DRI MultiDrug Urine Control 1, 25 mL
1600 DRI MultiDrug Urine Control 2, 10 mL
1555 DRI MultiDrug Urine Control 2, 25 mL

Precautions and Warnings

1. This test is for in vitro diagnostic use only. The components are harmful if swallowed.
2. Reagents used in the assay components contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with a large volume of water to prevent azide build-up.
3. Do not use the reagents beyond their expiration dates.

Reagent Preparation and Storage

The reagents are ready for use. No reagent preparation is required. All assay components, when stored properly at 2-8°C, are stable until the expiration date indicated on the label.

Specimen Collection and Handling

Collect urine specimens in plastic or glass containers. Testing of fresh urine specimens is suggested.

The Mandatory Guidelines for Federal Workplace Drug Testing Programs; Final Guidelines recommends that specimens that do not receive an initial test within 7 days of arrival in the laboratory should be placed into secure refrigeration units.

Samples within a pH range of 3 to 11 are suitable for testing with this assay.

An effort should be made to keep pipetted samples free of gross debris; it is recommended that highly turbid specimens be centrifuged before 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.

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

Quality Control and Calibration

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 the established range. If results fall outside of the established range, assay results are invalid.

Qualitative analysis
For qualitative analysis of samples, use the 300 ng/mL calibrator as a cutoff level. The DRI® MultiDrug Urine Calibrator 2, which contains 300 ng/mL benzoylecgonine, is used as a cutoff reference for distinguishing “positive” and “negative” samples.

Semiquantitative analysis
For semiquantitative analysis, use all calibrators.

Results and Expected Values

Qualitative results
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.

Semiquantitative results
When a rough estimate of cocaine metabolites concentration is required, a calibration curve can be established with all calibrators. The concentration of the sample can be estimated by quantitation off the calibration curve. When the sample concentration is greater than the highest calibrator, it may be diluted and retested.

Limitations

1. A positive result from this assay indicates only the presence of cocaine metabolites and does not necessarily correlate with the extent of physiological and psychological effects.
2. A positive result by this assay should be confirmed by an other 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 (eg, technical or procedural) not listed in the specificity table may interfere with the test and cause false results.

Specific Performance Characteristics

Precision

The Negative, 225 ng/mL, 300 ng/mL, 375 ng/mL were assayed with a Hitachi 717 analyzer. The following results were obtained:

	Within-run (n=20)		**Run-to-run (n=20)**	
Calibrator or Control	Mean ± SD (mA/min)	% CV	Mean ± SD (mA/min)	% CV
Negative	302 ± 2.0	0.7	302 ± 3.9	1.3
225 ng/mL	341 ± 2.5	0.7	342 ± 3.9	1.1
300 ng/mL	354 ± 3.4	0.9	354 ± 4.9	1.4
375 ng/mL	374 ± 2.4	0.6	374 ± 5.1	1.4

Accuracy

Two hundred and nine clinical specimens were tested with both DRI Cocaine Metabolite Assay and a commercially available cocaine metabolite assay. One hundred and four were tested positive and one hundred and five were tested negative by both assays. In addition, all positive specimens were confirmed by GC/MS to contain cocaine metabolites.

Sensitivity

Sensitivity, defined as the lowest concentration that can be differentiated from the negative urine calibrator with 95% confidence, is 40 ng/mL.

Specificity

Benzoylecgonine, cocaine and other compounds that are concurrently present in the urine were tested for cross- reactivity in the assay. The following table summarizes the results obtained at the concentrations tested for each potential cross-reactant.

Compound	**Concentration Tested (µg/mL)**	**Result**
Benzoylecgonine	0.3	positive
Cocaine	50	positive
Ecgonine	100	positive
Acetaminophen	1000	negative
Acetylsalicylic acid	1000	negative
Amphetamine	1000	negative
Amobarbital	1000	negative
Benzocaine	1000	negative
Caffeine	100	negative
Chlorpromazine	500	negative
Codeine	1000	negative
Dextromethorphan	100	negative
Ecgonine Methyl Ester	100	negative
Lidocaine	1000	negative
Meperidine	1000	negative
Methadone	1000	negative
Morphine	200	negative
Oxazepam	100	negative
Phencyclidine	1000	negative
Phenobarbital	1000	negative
Promethazine	100	negative
Propoxyphene	1000	negative
Secobarbital	1000	negative

References

1. *Urine Testing for Drug of Abuse*. National Institute on Drug Abuse (NIDA) Research Monograph 73, 1986.
2. Mandatory Guidelines for Federal Workplace Drug Testing Program. National Institute on Drug Abuse. *Federal Register* Vol. **53**, No 69, pp 11970 (1988).
3. Rubenstein KE, Schneider RS, and EF Ullman: Homogeneous enzyme immunoassay: a new immunochemical technique. *Biochem Biophys Res Commun* 47:846-851 (1972).

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