DRI® Cotinine Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use**

Rx Only

REF 10018516 (3 x 18 mL Kit)
0394 (100 mL Kit)
0395 (500 mL Kit)

Intended Use
The DRI® Cotinine Assay is an in vitro diagnostic medical device intended for the qualitative and semiquantitative determination of Cotinine in human urine at a cutoff level of 500 ng/mL. This assay is intended as an aid in the detection of cotinine after use or exposure to tobacco products.

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) or Liquid chromatography/tandem mass spectrometry (LC-MS/MS) is the preferred confirmatory method.

Summary and Explanation of the Test
Tobacco smoking or passive inhalation of tobacco smoke results in the absorption of nicotine through the lungs and mucus membranes of the mouth. When nicotine is absorbed, it is readily metabolized into cotinine as its major metabolite.[1,2,3] Cotinine is detectable in the urine of smokers even several days after the termination of smoking.

Several methods, including the measurement of thiocyanate, carbon monoxide and cotinine, can be used to determine an individual's smoking status.[4,5,6] Measurement of either carbon monoxide or thiocyanate can give a false indication of tobacco use, as they may come from other environmental sources.[7] Cotinine, on the other hand, can only be derived from the metabolism of nicotine, and is therefore the best indicator of smoking status.[8]

The DRI Cotinine Assay is a liquid, ready-to-use homogeneous enzyme immunoassay. The assay is based on competition between cotinine labeled with glucose-6-phosphate dehydrogenase (G6PDH) enzyme and free cotinine in the sample for a fixed amount of cotinine-specific antibody binding sites. 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 cotinine antibody, glucose-6-phosphate (G6P) and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.
Enzyme Conjugate Reagent:
Contains cotinine labeled with glucose-6-phosphate dehydrogenase (G6PDH) in Tris buffer with sodium azide as preservative.

Additional Materials Required (sold separately):

REF	Kit Description
0404	DRI Cotinine Calibrator Kit, 6 x 5 mL
0460	DRI Cotinine Low Control, 5 mL
0470	DRI Cotinine High Control, 5 mL

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

Reagents used in the assay components contain ≤0.09% sodium azide. Avoid contact with skin and mucous membranes. Refer to SDS for additional precautions, handling instructions, and accidental exposure treatment.

DANGER: The reagents contain ≤0.2% bovine serum albumin (BSA) and ≤0.5% Mouse monoclonal anti-cotinine antibody. Avoid inhalation. May cause skin or inhaled allergic reaction. Refer to 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.

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 refrigerated, are stable until the expiration date indicated on the label.

In the case of accidental spill, clean and dispose of material according to your laboratory's SOP, local, and state regulations.

In the case of damaged packaging on arrival, contact your technical support representative (refer to back page of this PI).

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[9] of arrival at the laboratory may be placed into a secure refrigeration unit at 2 to 8°C for 14 days.[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.

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[12]
Qualitative analysis
For qualitative analysis of samples, use the 500 ng/mL calibrator as a cutoff level. The DRI Cotinine Calibrator, which contains 500 ng/mL cotinine, is used as a cutoff reference for distinguishing "positive" and "negative" samples.

Semiquantitative 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 your laboratory. 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.

Results and Expected Values
Qualitative results
The 500 ng/mL calibrator is 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.

Semiquantitative results
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. This method can be used to determine dilutions for confirmation or for quality control purposes. When the estimated samples concentration is greater than the highest calibrator the sample can be diluted in Negative Calibrator and retested. The assay range is from 300 ng/mL to 2000 ng/mL.

Limitations
1. A positive result from this assay indicates only the presence of cotinine 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 non-immunological 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, e.g., technical, procedural issues or other cotinine-like compounds other than those investigated in the specificity study, may interfere with the test and cause false results.

Typical Performance Characteristics

Typical performance results obtained on the Hitachi 717 analyzer are shown below.[13] The results obtained in your laboratory may differ from these data.

Precision

Negative control, positive control and cutoff calibrator were tested using a modified NCCLS protocol. The test was run in qualitative and semiquantitative modes by testing all three levels in replicates of 6, twice per day for 10 days.

Qualitative (mA/min)

Calibrator/ Control	Within-run Precision			Total Precision		
	Mean	SD	%CV	Mean	SD	%CV
Low Control	404	2.2	0.5	404	2.7	0.7
Cutoff Calibrator	421	2.3	0.5	421	2.9	0.7
High Control	437	2.6	0.6	437	3.5	0.8

Semiquantitative (ng/mL)

Calibrator/ Control	Within-run Precision			Total Precision		
	Mean	SD	%CV	Mean	SD	%CV
Low Control	333	20.0	6.0	333	32.0	9.4
Cutoff Calibrator	510	27.9	5.5	510	38.6	7.6
High Control	716	36.4	5.1	716	50.4	7.0

Accuracy

One hundred and ninety-four samples were analyzed by the DRI Cotinine Assay and OTI AUTO-LYTE® Cotinine EIA using 500 ng/mL Cotinine as cutoff calibrator. All the samples were confirmed by GC/MS. The results obtained by both qualitative and semiquantitative modes are summarized below:

Qualitative

Out of 194 samples, 112 samples were detected as positive and 60 samples as negative by both immunoassays.

AUTO-LYTE® Cotinine EIA	DRI Cotinine Assay +	DRI Cotinine Assay -
+	112	21*
-	1*	60

GC/MS	DRI Cotinine Assay +	DRI Cotinine Assay -
+	110	0
-	3**	81

* GC/MS confirmed the result obtained by the DRI Cotinine Assay.
** Two out of the three discrepant samples were borderline positive.

Semiquantitative

Out of 194 samples, 114 samples were detected as positive and 63 as negative by both immunoassays.

AUTO-LYTE® Cotinine EIA	DRI Cotinine Assay +	DRI Cotinine Assay -
+	114	17*
-	0	63

GC/MS	DRI Cotinine Assay +	DRI Cotinine Assay -
+	110	0
-	4**	80

* GC/MS confirmed the result obtained by the DRI Cotinine Assay.
** Two out of the four discrepant samples were borderline positive.

Sensitivity

Sensitivity, defined as the lowest concentration that can be differentiated from the negative urine calibrator with 95% confidence, is 34 ng/mL.

Specificity

Compounds structurally related to cotinine were tested for cross-reactivity. Results are listed below:

Compound	Concentration (µg/mL)	Result
3-Hydroxy-Cotinine	12.5	Positive
Niacinamide	500	Negative
Nicotine	500	Negative
Nicotinic Acid	500	Negative
Nicotinic Acid N-Oxide	500	Negative

Compounds structurally unrelated to cotinine produced negative results at the concentrations listed below:

Compound	Concentration (µg/mL)
Acetaminophen	1000
Acetylsalicylic acid	1000
Amitriptyline	50
Amphetamine	1000
Benzoylecgonine	1000
Caffeine	100
Codeine	1000
Diazepam	100
Doxylamine	500
Ephedrine	1000
Ibuprofen	500
Phenytoin	40
d-Methamphetamine	100
l-Methamphetamine	100
Morphine	1000
Nortriptyline	50
Oxazepam	500
Phencyclidine	500
Pheniramine	50
Phenobarbital	1000
Primidone	50
Propoxyphene	1000
Quinidine	200
Ranitidine	500
Secobarbital	1000
Theophylline	50
11-Nor-Δ^9-THC-9-COOH	10
Valproic Acid	150
Uric Acid	200

Interference

Endogenous and exogenous substances were studied for potential interference with the Cotinine Assay. No interference was observed in urine samples containing compounds at the concentrations listed below. Urine pH was also studied for possible interference.

Compound	Concentration
Acetaminophen	100 µg/mL
Acetone	1000 mg/dL
Ascorbic Acid	1250 mg/dL
Aspirin	100 µg/mL
Caffeine	100 µg/mL
Creatinine	500 mg/dL
Ethanol	1 g/dL
Galactose	10 mg/dL
Gamma Globulin	500 mg/dL
Glucose	3000 mg/dL
Hemoglobin	300 mg/dL
Human serum Albumin	500 mg/dL
Ibuprofen	100 µg/mL
Oxalic Acid	100 mg/dL
Riboflavin	7.5 mg/dL
Sodium Chrloride	0.5 g/dL
Urea	0.8 g/dL
pH range	4-9

References

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10. C52-A2, Toxicology and Drug Testing in the Clinical Laboratory; Approved Guideline – Second Edition, *Clinical and Laboratory Standards Institute (CLSI)* (April 2007).
11. *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.
12. Data on traceability are on file at Microgenics Corporation, a part of Thermo Fisher Scientific.
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Glossary:
http://www.thermofisher.com/symbols-glossary

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