IMMULITE® 2000

Nicotine Metabolite

DPC®

IMMULITE® 2000 Nicotine Metabolite

English

Intended Use: *For research use only* with the IMMULITE 2000 Analyzer. *Not for use in diagnostic procedures.*

Catalog Number: **L2KNM2** (200 tests), **L2KNM6** (600 tests)

Test Code: **NM** Color: **Dark Green**

CDC Test System Identifier Code: 10418
CLIA Complexity Category: Moderate

Summary and Explanation

The dried leaves of the tobacco plant *Nicotiana tabacum* are the only significant source of nicotine in man. When the leaves are smoked (in cigarettes, pipes, cigars), nasally insufflated (as snuff), or chewed, nicotine is rapidly absorbed and distributed widely.[6] Metabolism is extensive, mainly by oxidation to cotinine, nicotine-*N*-oxide, and a number of other (mostly unidentified) metabolites.[1,2,7,8]

The detection of smoking by measurement of nicotine metabolites such as cotinine is favored over other methods, like carboxyhemoglobin or thiocyanate determinations.[1,3,9] This is partly because cotinine and other nicotine metabolites are tobacco specific, whereas carboxyhemoglobin and thiocyanate may be present as the result of exposure to other environmental factors. Moreover, cotinine and other nicotine metabolites have a half-life in blood of more than 10 hours,[1] while nicotine itself and carboxyhemoglobin have much shorter half lives.[5,9]

Principle of the Procedure

IMMULITE 2000 Nicotine Metabolite is a solid-phase competitive chemiluminescent Immunoassay.

Incubation Cycles: 1 × 30 minutes.

Specimen Collection

Serum: Centrifuging serum samples before a complete clot forms may result in the presence of fibrin.

The use of an ultracentrifuge is recommended to clear lipemic samples.

Hemolyzed samples may indicate mistreatment of a specimen before receipt by the laboratory.

Blood collection tubes from different manufacturers may yield differing values, depending on materials and additives, including gel or physical barriers, clot activators and/or anticoagulants.

Urine: Collect urine without preservatives. The urine should be cleared by filtration or centrifugation before use. Remove the supernatant urine from the sediment and mix by *gentle* swirling or inversion.

Volume Required: 20 µL sample (serum or urine).

Storage: 30 days at 2–8°C. For longer storage, freeze at –20°C.
Avoid repeated freeze/thaw cycles.

Dilution Factor: 40.
For positive urine samples, select 40 in the Dilution Factor window.

Warnings and Precautions

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Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water.

Materials Supplied

Components are a matched set. Labels on the inside box are needed for the assay.

Nicotine Metabolite Bead Pack (L2NM12)
With barcode. 200 beads, coated with polyclonal rabbit anti-cotinine antibody. Stable at 2–8°C until expiration date.
L2KNM2: 1 pack. **L2KNM6:** 3 packs.

Nicotine Metabolite Reagent Wedge (L2NMA2)
With barcode. 11.5 mL alkaline phosphatase (bovine calf intestine) conjugated to cotinine in buffer, with preservative. Stable at 2–8°C until expiration date.
L2KNM2: 1 wedge. **L2KNM6:** 3 wedges.

Before use, tear off the top of the label at the perforations, without damaging the barcode. Remove the foil seal from the top of wedge; snap the sliding cover down into the ramps on the reagent lid.

Nicotine Metabolite Adjustors (LNML, LNMH)
Two vials (Low and High), 2 mL each, containing cotinine in processed human serum, with preservative. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.
L2KNM2: 1 set. **L2KNM6:** 2 sets.

Before making an adjustment, place the appropriate Aliquot Labels (supplied with the kit) on test tubes so that the barcodes can be read by the on-board reader.

Kit Components Supplied Separately

Multi-Diluent 2 (L2M2Z, L2M2Z4)
For the on-board dilution of patient urine samples. One vial, concentrated (ready-to-use), nonhuman protein/buffer matrix, with preservative. Storage: 30 days (after opening) at 2–8°C or 6 months (aliquotted) at –20°C.
L2M2Z: 25 mL **L2M2Z4:** 55 mL

Barcode labels are provided for use with the diluent. Before use, place an appropriate label on a 16 × 100 mm test tube, so that the barcodes can be read by the on-board reader.
L2M2Z: 3 labels. **L2M2Z4:** 5 labels.

L2SUBM: Chemiluminescent Substrate
L2PWSM: Probe Wash
L2KPM: Probe Cleaning Kit
LRXT: Reaction Tubes (disposable)
L2ZT: 250 Sample Diluent Test Tubes (16 × 100 mm)
L2ZC: 250 Sample Diluent Tube Caps

SDCM: Bi-level, human serum-based control.

Also required
Distilled or deionized water; test tubes; controls.

Assay Procedure

See the IMMULITE 2000 Operator's Manual for: preparation, setup, dilutions, adjustment, assay and quality control procedures.

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in the IMMULITE 2000 Operator's Manual.

Recommended Adjustment Interval: 2 weeks.

Quality Control Samples: Use controls or sample pools with at least two levels (low and high) of cotinine.

Expected Values

Each laboratory should establish its own reference ranges.

Limitation

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.]

Performance Data

See Tables and Graphs for data *representative* of the assay's performance. Results are expressed in ng/mL. (Unless otherwise noted, all were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

Calibration Range: 10 – 500 ng/mL

Analytical Sensitivity: 5 ng/mL

Linearity: Samples were assayed under various dilutions. (See "Linearity" table for representative data.)

Recovery: Samples spiked 1 to 19 with three cotinine solutions (700, 3,000 and 6,000 ng/mL) were assayed. (See "Recovery" table for representative data.)

Specificity: The antibody used is highly specific for cotinine, with nondetectable crossreactivity to other naturally occurring compounds or therapeutic drugs that might be present in patient samples. The listed compounds were found nondetectable when tested at a concentration of 25,000 ng/mL. (See “Specificity ” table.)

References

1) Benowitz NL, et al. Cotinine disposition and effects. Clin Pharmacol Ther 1983;34:604-11. 2) Frankenburg WG, Vaitekunas AA. The chemistry of tobacco fermentation. I. Conversion of the alkaloids; identification of cotinine in fermented leaves. J Am Chem Soc 1957;79:149-51. 3) Haley NJ, Axelrad C, Tilton K. Validation of self-reported smoking behavior: biochemical analyses of cotinine and thiocyanate. Am J Public Health 1983;73:1204-7. 4) Johnston C. Cigarette smoking and the outcome of human pregnancies; a status report on the consequences. Clin Toxicol 1981;18:189-209. 5) Kyerematen GA, et al. Smoking-induced changes in nicotine and its metabolites. Clin Pharmacol Ther 1982;32:769-80. 6) Larsson I, et al. Nicotine and cotinine levels in different body fluids of smokers. Acta Pharmacol Toxicol 1986;Suppl. 5:312. 7) Matsukara S, et al. Cotinine excretion and daily cigarette smoking in habituated smokers. Clin Pharmacol Ther 1979;25:555-61. 8) Pinner A. Ueber nicotin. Arch Pharmacol 1893;231:378-448. 9) Pojer R, et al. Carboxyhemoglobin, cotinine, and thiocyanate assay compared for distinguishing smokers from non-smokers. Clin Chem 1984;30:1377-80. 10) U.S. Department of Health and Human Services. The health consequences of smoking: cancer. DHHS Publication Number (PHS) 82-50179; 1982. 11) U.S. Department of Health and Human Services. The health consequences of smoking: cardiovascular disease. DHHS Publication Number (PHS) 84-50204; 1983. 12) Svensson CK. Clinical pharmacokinetics of nicotine. Clin Pharmacokinet 1987;12:30-40. 13) Perkins SL, et al. High-performance liquid-chromatographic method compared with a modified radioimmunoassay of cotinine in plasma. Clin Chem 1991;37:1989-93. 14) National Committee for Clinical Laboratory Standards. Procedures for the collection of diagnostic blood specimens by venipuncture; approved standard. 4th ed. NCCLS Document H3-A4, Wayne, PA: NCCLS, 1998.

Technical Assistance

In the United States**, available for research use only.** Contact DPC's Technical Services department.
Tel: 800.372.1782 or 973.927.2828
Fax: 973.927.4101. Outside the United States, contact your National Distributor.

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Tables and Graphs

Linearity (ng/mL)

	Dilution	Observed	Expected	%O/E
1	8 in 8	118	—	—
	4 in 8	56	59	95%
	2 in 8	33	30	110%
	1 in 8	15.6	14.5	105%
2	8 in 8	256	—	—
	4 in 8	120	128	94%
	2 in 8	60	64	94%
	1 in 8	31	32	97%
3	8 in 8	302	—	—
	4 in 8	146	151	97%
	2 in 8	66	76	87%
	1 in 8	34	38	89%
4	8 in 8	375	—	—
	4 in 8	196	188	104%
	2 in 8	99	94	105%
	1 in 8	47	47	100%
5	8 in 8	450	—	—
	4 in 8	234	225	104%
	2 in 8	122	113	108%
	1 in 8	59	56	105%

Recovery (ng/mL)

	Solution	Observed	Expected	%O/E
1	—	ND	—	—
	A	32	35	91%
	B	162	150	108%
	C	316	300	105%
2	—	30	—	—
	A	64	64	100%
	B	178	179	99%
	C	311	329	95%
3	—	54	—	—
	A	93	86	108%
	B	204	201	101%
	C	280	351	80%
4	—	74	—	—
	A	109	105	104v
	B	196	220	89%
	C	333	370	90%
5	—	123	—	—
	A	180	152	118%
	B	274	267	103%
	C	432	417	104%

Specificity

Compound	ng/mL Added	Apparent ng/mL	% Cross-reactivity
Acetylsalicylic acid	25,000	ND	ND
Barbituric acid	25,000	ND	ND
Benzoylecgonine	25,000	ND	ND
Butalbital	25,000	ND	ND
Caffeine	25,000	ND	ND
Codeine	25,000	ND	ND
Dextromethorphan	25,000	ND	ND
Diazepam	25,000	ND	ND
Fentanyl	25,000	ND	ND
Theobromine	25,000	ND	ND
L-Lobeline	25,000	ND	ND
Methadone	25,000	ND	ND
Morphine	25,000	ND	ND
Morphine-3-glucuronide	25,000	ND	ND
Nicotinic acid	25,000	ND	ND
Oxazepam	25,000	ND	ND
Phenobarbital	25,000	ND	ND
Secobarbital	25,000	ND	ND
Buprenorphine	25,000	ND	ND
Niacinamide	25,000	ND	ND

ND: not detectable.

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2004-07-29

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