Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

OneStep Methylphenidate RapiCard™ InstaTest

REF 121149-1-44

IVD See external Label 2-30°C ∑=1 Test

A rapid test for the qualitative detection of ritalinicacidin human urine.
For medical and other professional in vitro diagnostic use only.

INTENDED USE

The MPD RapiCard™ (Urine) is a rapid chromatographic immunoassay for the detection of *ritalinic acid* in urine at a cut-off concentration of 1000ng/ml. This test will detect other related compounds, please refer to the Analytical Specificity table in this package insert.
This assay provides only a qualitative, preliminary analytical test result. A more specific alternate 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 positive results are used.

SUMMARY AND EXPLANATION

Methylphenidate (tradenames Concerta, Methylin, Medikinet, Ritalin, Equasym XL, Quillivant XR, Metadate) is acentral nervous system(CNS) stimulant of the phenethylamine[1] and piperidine classes that is used in the treatment of attention deficit hyperactivity disorder (ADHD),postural orthostatic tachycardia syndrome and narcolepsy. Methylphenidate has been studied and researched for over 50 years and has a very good efficacy and safety record for the treatment of ADHD.[2] It was first licensed by the U.S. Food and Drug Administration (FDA) in 1955 for treating what was then known as hyperactivity. Prescribed to patients beginning in 1960, the drug has become increasingly heavily prescribed since the 1990s, when the diagnosis of ADHD itself became more widely accepted. [3]
ADHD and other similar conditions are believed to be linked to sub-performance of the dopamine and norepinephrine functions in the brain, primarily in the prefrontal cortex, responsible for self-regulatory function (e.g., inhibition, motivation, and memory) and executive function (e.g., reasoning, organizing, problem solving, and planning).[4][5]Methylphenidate's mechanism of action involves the inhibition of catecholamine reuptake, primarily as a dopamine reuptake inhibitor. Methylphenidate acts by blocking the dopamine transporter and norepinephrine transporter, leading to increased concentrations of dopamine and norepinephrine within the synaptic cleft. This effect in turn leads to increased neurotransmission of dopamine and norepinephrine. Methylphenidate is also a 5HT1A receptor agonist.[6]
Methylphenidate taken orally has a bioavailability of 11-52% with duration of action around 1-4 hours for instant release, 3–8 hours for sustained release, and 8–12hours for extended release (Concerta) [7]. The major metabolite of methylphenidate is ritalinic acid and methylphenidate; the metabolite of 80% had excreted by urine within 24 hours, the concentration of ritalinic acid min after administration was eight times higher than that of methylphenidate [8].
The MPD RapiCard™ (Urine) is a rapid urine-screening test that can be performed without the use of an instrument. The test utilizes the antibody to selectively detect elevated levels of ritalinic acid in urine. The MPD RapiCard™ (Urine) yields a positive result when the ritalinic acid in urine exceeds the cut-off level.

TEST PRINCIPLE

The MPD RapiCard™ (Urine) is an immunoassay based on the principle of competitive binding. Drugs which may be present in the urine specimen compete against the drug conjugate for binding sites on the antibody.
During testing, a urine specimen migrates upward by capillary action. ritalinic acid, if present in the urine specimen below the cut-off level, will not saturate the binding sites of the antibody in the test. The antibody coated particles will then be captured by immobilized Methylphenidate-protein conjugate and a visible colored line will show up in the test line region. The colored line will not form in the test line region if the ritalinic acid level exceeds the cut-off level, because it will saturate all the binding sites of anti-Methylphenidate antibody.
A drug-positive urine specimen will not generate a colored line in the test line region because of drug competition, while a drug-negative urine specimen or a specimen containing a drug concentration less than the cut-off will generate a line in the test line region. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test contains mouse monoclonal anti-Methylphenidate antibody coupled particles and Methylphenidate -protein conjugate. A goat antibody is employed in the control line system.

SPECIMEN COLLECTION AND PREPARATION

Urine Assay
The urine specimen must be collected in a clean and dry container. Urine collected at any time of the day may be used. Urine specimens exhibiting visible particles should be centrifuged, filtered, or allowed to settle to obtain a clear specimen for testing.
Specimen Storage
Urine specimens may be stored at 2-8°C for up to 48 hours prior to testing. For prolonged storage, specimens may be frozen and stored below -20°C. Frozen specimens should be thawed and mixed before testing.

MATERIALS AND COMPONENTS

Materials provided with the test kits

- Test Cassettes
- Droppers
- Package insert

Materials required but not provided

- Specimen collection container
- Timer

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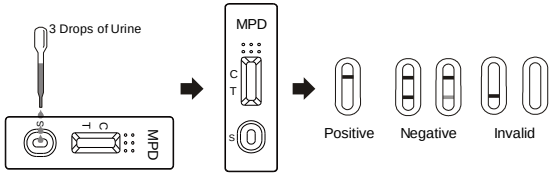
ASSAY PROCEDURE

Allow the test, urine specimen, and/or controls to reach room temperature (15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it within one hour.
2. Place the test device on a clean and level surface. Hold the dropper vertically and **transfer 3 full drops of urine** (approx. 120 µL) to the specimen well (S) of the test cassette, and then start the timer. Avoid trapping air bubbles in the specimen well (S). See the illustration below.
3. Wait for the colored line(s) to appear. Read results at 5 minutes. Do not interpret the result after 10 minutes.



RESULTS

(Please refer to the illustration above)

NEGATIVE:* Two lines appear. One colored line should be in the control line region (C), and another apparent colored line should be in the test line region (T). This negative result indicates that the ritalinic acid concentration is below the detectable cut-off level.

***NOTE:** The shade of color in the test line region (T) may vary, but it should be considered negative whenever there is even a faint colored line.

POSITIVE: One colored line appears in the control line region (C). No line appears in the test line region (T). This positive result indicates that the ritalinic acid concentration exceeds the detectable cut-off level.

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

PERFORMANCE CHARACTERISTICS

Accuracy

A side-by-side comparison was conducted using The MPD RapiCard™ (Urine) and GC/MS at the cut-off of 1000ng/ml. Testing was performed on 100 clinical specimens previously collected from subjects present for Drug Screen Testing. The following results were tabulated:

Method		GC/MS		Total Results
MPD Rapid Test Cassette	Results	Positive	Negative	
	Positive	34	1	35
	Negative	3	62	65
Total Results		37	37	63
% Agreement		91.9%	98.4%	96.0%

Analytical Sensitivity

A drug-free urine pool was spiked with ritalinic acid at the following concentrations: 0ng/ml, 500ng/ml, 750ng/ml, 1000ng/ml, 1250ng/ml, 1500ng/ml and 3000ng/ml. The result demonstrates >99% accuracy at 50% above and 50% below the cut-off concentration. The data are summarized below:

Ritalinic Acid Concentration (ng/ml)	Percent of Cut-off	n	Visual Result	
			Negative	Positive
0	0	30	30	0
500	-50%	30	30	0
750	-25%	30	27	3
1000	Cut-off	30	16	14
1250	+25%	30	6	24
1500	+50%	30	0	30
3000	3X	30	0	30

Analytical Specificity

The following table lists compounds that are positively detected in urine by the MPD RapiCard™ (Urine) at 5 minutes.

Compound	Concentration (ng/mL)
Ritalinic Acid	1000
Methylphenidate (Ritalin)	350

Precision

A study was conducted at three hospitals by laypersons using three different lots of product to demonstrate the within run, between run and between operator precision. An identical panel of coded specimens containing, according to GC/MS, no ritalinic acid, 25% ritalinic acid above and below the cut-off and 50% ritalinic acid above and below the 1000ng/ml cut-off was provided to each site. The following results were tabulated:

Ritalinic Acid Concentration (ng/ml)	n per Site	Site A		Site B		Site C	
		-	+	-	+	-	+
0	10	10	0	10	0	10	0
500	10	10	0	10	0	10	0
750	10	9	1	9	1	9	1
1250	10	2	8	2	8	2	8
1500	10	0	10	0	10	0	10

Effect of Urinary Specific Gravity

Fifteen urine specimens of normal, high, and low specific gravity ranges were spiked with 500ng/ml and 1500ng/ml of ritalinic acid. The MPD RapiCard™ (Urine) was tested in duplicate using the fifteen neat and spiked urine specimens. The results demonstrate that varying ranges of urinary specific gravity do not affect the test results.

Effect of Urinary pH

The pH of an aliquoted negative urine pool was adjusted to a pH range of 5 to 9 in 1 pH unit increments and spiked with ritalinic acid to 500ng/ml and 1500ng/ml. The spiked, pH-adjusted urine was tested with The MPD RapiCard™ (Urine) in duplicate. The results demonstrate that varying ranges of pH do not interfere with the performance of the test.

Cross-Reactivity

A study was conducted to determine the cross-reactivity of the test with compounds in either drug-free urine or ritalinic acid positive urine. The following compounds show no cross-reactivity when tested with The MPD RapiCard™ (Urine) at a concentration of 100 µg/ml.

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Non Cross-Reacting Compounds

Acetone	Dopamine	Oxalic Acid
Albumin	(+/-)-Epinephrine	Penicillin-G
Ampicillin	Erythromycin	Pheniramine
Ascorbic	Acid Ethanol	Phenothiazine
Aspartame	Furosemide	L-Phenylephrine
Aspirin	Glucose	β-Phenylethylamine
Atropine	Guaiacol Glyceryl Ether	Procaine
Benzocaine	Hemoglobin	Quinidine
Bilirubin	Ibuprofen	Ranitidine
Caffeine	(+/-)-Isoproterenol	Riboflavin
Chloroquine	Ketamine	Sodium Chloride
(+)-Chlorpheniramine	Levorphanol	Sulindac
(+/-)-Chlorpheniramine	Lidocaine	Tyramine
Creatine	(+)-Naproxen	4-Dimethylaminoantipyrine
Dexbrompheniramine	Niacinamide	(1R,2S)-(-)-N-Methyl-Ephedrine
Dextromethorphan	Nicotine	
Diphenhydramine	(+/-)-Norephedrine	

QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as good laboratory testing practice to confirm the test procedure and to verify proper test performance.

LIMITATION OF PROCEDURE

1. The MPD RapiCard™ (Urine) provides only a qualitative, preliminary analytical result. A secondary analytical method must be used to obtain a confirmed result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.
2. It is possible that technical or procedural errors, as well as other interfering substances in the urine specimen may cause erroneous results.
3. Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results regardless of the analytical method used. If adulteration is suspected, the test should be repeated with another urine specimen.
4. A positive result indicates presence of the drug or its metabolites but does not indicate level of intoxication, administration route or concentration in urine.
5. A negative result may not necessarily indicate drug-free urine. Negative results can be obtained when drug is present but below the cut-off level of the test.
6. Test does not distinguish between drugs of abuse and certain medications.

EXPECTED VALUES

This negative result indicates that the ritalinic acid concentration is below the detectable level of 1000ng/ml. Positive result means the concentration of ritalinic acid is above the level of 1000ng/ml. The MPD RapiCard™ has a sensitivity of 1000ng/ml

PRECAUTION

- For medical and other professional *in vitro* diagnostic use only. Do not use after the expiration date.
- The test should remain in the sealed pouch until use.
- All specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The used test should be discarded according to local regulations.

STORAGE

Store as packaged in the sealed pouch either at room temperature or refrigerated (2-30°C). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

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