

ARK™ Methylphenidate Metabolite Assay

This ARK Diagnostics, Inc. package insert for the ARK Methylphenidate Metabolite Assay must be read prior to use. Package insert instructions must be followed accordingly. The assay provides a simple and rapid analytical screening procedure for detecting Methylphenidate Metabolite in urine. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Report any serious incident that has occurred in relation to the device to the manufacturer and the appropriate competent authority as applicable.

Customer Service

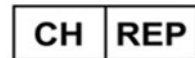


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





EC REP

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Key to Symbols Used

	Batch code	 YYYY-MM-DD	Use by/Expiration date
	Catalog Number		Manufacturer
	Authorized Representative		CE Mark with notified body number
	Consult Instructions for Use		Reagent 1 / Reagent 2
	Temperature limitation		In Vitro Diagnostic Medical Device
Rx Only	For Prescription Use Only		

1 Name

ARK™ Methylphenidate Metabolite Assay

2 Intended Use

The ARK Methylphenidate Metabolite Assay is an immunoassay intended for the qualitative and/or semiquantitative determination of Methylphenidate Metabolite in human urine at a cutoff concentration of 100 ng/mL. The assay is intended for use in laboratories with automated clinical chemistry analyzers. This *in vitro* diagnostic device is for prescription use only.

The semiquantitative mode is for the purpose of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method, such as Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS), or (2) permitting laboratories to establish quality control procedures.

The ARK Methylphenidate Metabolite 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) or Liquid Chromatography/tandem Mass Spectrometry (LC-MS/MS) is the preferred confirmatory method. Clinical consideration and professional judgment should be exercised with any drug test result, particularly when the preliminary test result is positive.

3 Summary and Explanation of Test

Methylphenidate (Ritalin®) is a mild central nervous system stimulant used for the treatment of Attention Deficit Hyperactivity Disorder (ADHD)¹. Methylphenidate, a Schedule II substance under the United States Controlled Substances Act², has a high potential for abuse because of its pharmacological properties which are similar to those of amphetamines and cocaine³. Methylphenidate is primarily metabolized through de-esterification to ritalinic acid (methylphenidate metabolite) with 80% of the dose excreted in urine as ritalinic acid^{3,4}, and less than 11% excreted in urine as unchanged methylphenidate^{4,5}.

4 Principles of the Procedure

The ARK Methylphenidate Metabolite Assay is a homogeneous enzyme immunoassay technique used for the analysis of Methylphenidate Metabolite in human urine. The assay is based on competition between drug in the specimen and drug labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH) for antibody binding sites. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly related to the drug concentration. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH in the presence of glucose-6-phosphate (G6P), resulting in an absorbance change that is measured spectrophotometrically. Endogenous G6PDH does not interfere because the coenzyme NAD functions only with the bacterial enzyme used in the assay.

5 Reagents

REF	Product Description	Quantity/Volume
5042-0001-00	ARK Methylphenidate Metabolite Assay Reagent [R1] – Antibody/Substrate rabbit polyclonal antibodies to Methylphenidate Metabolite, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, sodium azide, and stabilizers	1 X 28 mL
	Reagent [R2] – Enzyme Methylphenidate Metabolite derivative labeled with recombinant glucose-6-phosphate dehydrogenase (rG6PDH), bovine serum albumin, buffer, sodium azide and stabilizers	1 X 14 mL

Reagent Handling and Storage

ARK Methylphenidate Metabolite Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

ARK Methylphenidate Metabolite products contain ≤0.09% sodium azide. As a precaution, affected plumbing including instrumentation should be flushed adequately with water to mitigate the potential accumulation of explosive metal azides. No special handling is required regarding other assay components.

6 Warnings and Precautions

- For *In Vitro* Diagnostic Use. For prescription use only.
- Reagents [R1] and [R2] are provided as a matched set and should not be interchanged with reagents from different lot numbers.
- Do not use reagents after the expiration date.
- Reagents contain ≤0.09% sodium azide.

7 Specimen Collection and Preparation for Analysis

- Each laboratory is responsible for supplying a valid specimen for analysis according to their quality procedures.
- Human urine is required. Treat as potentially infectious material.
- Collect urine using standard sampling cups and procedures. Care should be taken to preserve the chemical and physical integrity of the urine sample from the time it is collected until the time it is assayed, including during transport. Fresh urine specimens are suggested.
- Cap the urine sample immediately after collection, store refrigerated at 2–8°C (36–46°F) and assay within 7 days after collection. If the assay cannot be performed within 7 days, store the urine sample frozen at -20°C.⁶
- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.

- The presence of bubbles or foam on specimens can lead to short sample delivery and erroneous results.
- Frozen specimens must be thawed and mixed thoroughly prior to analysis.
- Centrifuge specimens with high turbidity or visible particulate matter before testing.
- Each laboratory should consult available literature and internal data regarding specimen stability. The recommended pH range for urine specimens is 4.0 – 11.0⁷.
- Obtain another sample for testing if adulteration of the sample is suspected. Adulteration of urine specimens can affect the test result.

8 Procedure

Materials Provided

ARK Methylphenidate Metabolite Assay – REF 5042-0001-00

Materials Required – Provided Separately

ARK Methylphenidate Metabolite Calibrator – REF 5042-0002-00

ARK Methylphenidate Metabolite Calibrator A (Negative) – REF 5042-0002-01

ARK Methylphenidate Metabolite Calibrator B (Cutoff) – REF 5042-0002-02

Quality Controls – ARK Methylphenidate Metabolite Control – REF 5042-0003-00

Instruments

Reagents R1 and R2 may need to be transferred to analyzer-specific reagent containers prior to use. Avoid cross-contamination of R1 and R2. Many automated clinical chemistry analyzers with photometric rate determination at 340 nm are suitable. Consult the analyzer-specific application sheet for programming the ARK Methylphenidate Metabolite Assay, available from your distributor or ARK Customer Service. Application Protocol Sheets bearing the CE Mark have been verified by the manufacturer. It is the responsibility of the laboratory to perform all appropriate validation for use of the assay with other settings or analyzers.

Refer to the instrument-specific operator's manual for daily maintenance.

Assay Sequence

To run or calibrate the assay, see the instrument-specific operator's manual.

Qualitative Results

Use the 100 ng/mL Calibrator B as a Cutoff Calibrator to distinguish negative and positive samples. Run the ARK Methylphenidate Metabolite Low (50 ng/mL) and High (150 ng/mL) Controls as Negative and Positive respectively. Report test results less than the response value for the Cutoff Calibrator as Negative. Report results equal to or greater than the response value for the Cutoff Calibrator as Positive.

Semiquantitative Results

Perform a 5-point calibration procedure; run calibrators in duplicate. Verify the calibration curve with the ARK Methylphenidate Metabolite Low (50 ng/mL) and

High (150 ng/mL) quality controls according to the established laboratory quality assurance plan. Specimens with sample results above the highest ARK Methylphenidate Metabolite calibrator level (1000 ng/mL) may be diluted in ARK Methylphenidate Metabolite Calibrator A (Negative urine) and retested.

When to Re-Calibrate

- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols
- A stored calibration curve was effective up to at least 8 days based on supporting data.

Quality Control (QC) and Calibration

Laboratories should establish QC procedures for the ARK Methylphenidate Metabolite Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Each laboratory should establish its own ranges for each new lot of controls. Control results should fall within established ranges as determined by laboratory procedures and guidelines. The ARK Methylphenidate Metabolite Control is intended for use in quality control of the ARK Methylphenidate Metabolite Assay.

In Qualitative Mode, the Low Control should be Negative and the High Control should be Positive relative to the 100 ng/mL Cutoff Calibrator.

9 Results and Expected Values

The actual Methylphenidate Metabolite concentration cannot be determined. A confirmatory method is required.

Qualitative Analysis – Negative Results

A specimen that gives a response value less than the ARK Methylphenidate Metabolite Calibrator B Cutoff response value is interpreted as negative; either the specimen does not contain Methylphenidate Metabolite or Methylphenidate Metabolite is present in a concentration below the cutoff level of this assay.

Qualitative Analysis – Positive Results

A specimen that gives a response value equal to or greater than the ARK Methylphenidate Metabolite Calibrator B Cutoff response value is interpreted as positive, indicating that Methylphenidate Metabolite is present.

Semiquantitative Analysis

The semiquantitation of positive results enables the laboratory to determine an appropriate dilution of the specimen for the confirmatory method. Semiquantitation also permits the laboratory to establish quality control procedures and assess reproducibility. Specimens with sample results above the highest ARK Methylphenidate Metabolite calibrator level (1000 ng/mL) may be diluted in ARK Methylphenidate Metabolite Calibrator A (Negative urine) and retested.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

10 Limitations

- The assay is designated for use with human urine only.
- ARK Methylphenidate Metabolite Assay reagents, calibrators and controls were developed as companion products. Performance with substituted products cannot be assured.
- A positive result using the ARK Methylphenidate Metabolite Assay indicates only the presence of Methylphenidate Metabolite and does not necessarily correlate with the extent of physiological and psychological effects.
- **Do not use Boric Acid as a preservative.**
- Interpretation of results must take into account that urine concentrations can vary extensively with fluid intake and other biological variables.
- It is possible that substances other than those tested in the specificity study may interfere with the test and cause false results.

11 Specific Performance Characteristics

The following performance characteristics were collected on the Beckman Coulter AU480® automated clinical chemistry analyzer using the ARK Methylphenidate Metabolite Assay.

Precision

Drug-free, negative human urine was supplemented with Methylphenidate Metabolite (0.0 to 200.0 ng/mL). Each level was assayed in quadruplicate twice a day for 20 days (N=160) in both qualitative and semiquantitative modes. Results are summarized in the tables below.

Qualitative Precision

Human Urine (ng/mL)	% Cutoff	# of Determinations	Results
0.0	-100	160	160 Negative
50.0	-50	160	160 Negative
75.0	-25	160	160 Negative
100.0	Cutoff	160	69 Negative/ 91 Positive
125.0	+25	160	160 Positive
150.0	+50	160	160 Positive
200.0	+100	160	160 Positive

Semiquantitative Precision

Human Urine (ng/mL)	Relative % Cutoff	# of Determinations	Mean (ng/mL)	Results	Repeatability (Within-Run Precision)		Within-Laboratory (Total Precision)	
					SD	%CV	SD	%CV
0.0	-100	160	1.2	160 Negative	1.22	NA	1.76	NA
50.0	-50	160	51.7	160 Negative	3.58	6.9	4.84	9.4
75.0	-25	160	74.8	160 Negative	4.59	6.1	6.35	8.5
100.0	Cutoff	160	99.6	92 Negative/ 68 Positive	5.37	5.4	7.48	7.5
125.0	+25	160	125.2	160 Positive	5.50	4.4	8.15	6.5

Human Urine (ng/mL)	Relative % Cutoff	# of Determinations	Mean (ng/mL)	Results	Repeatability (Within-Run Precision)		Within-Laboratory (Total Precision)	
					SD	%CV	SD	%CV
150.0	+50	160	149.2	160 Positive	7.03	4.7	9.31	6.2
200.0	+100	160	201.1	160 Positive	7.75	3.9	11.34	5.6

Analytical Recovery

Recovery across the assay range was assessed using the semiquantitative mode. Drug-free, negative human urine was supplemented with Methylphenidate Metabolite (1,250 ng/mL) and dilutions were made proportionally with drug-free human urine. Methylphenidate Metabolite concentrations ranged from 0.0 to 1000.0 ng/mL. At each level, percentage recovery was calculated based on the mean concentration (N=6) compared to the expected concentration. Results are summarized in the table below.

Theoretical Concentration (ng/mL)	Mean Concentration (ng/mL)	Recovery (%)
0.0	1.5	NA
25.0	23.8	95.1
50.0	47.8	95.6
100.0	99.8	99.8
200.0	195.1	97.6
400.0	386.5	96.6
600.0	563.3	93.9
800.0	756.1	94.5
1000.0	923.7	92.4

Analytical Specificity

The cross-reactivity of structurally related compounds was evaluated by spiking these compounds into drug-free, negative human urine and tested with the ARK Methylphenidate Metabolite Assay in both qualitative and semiquantitative modes. The compounds listed in the table below were negative at the concentrations tested with the ARK Methylphenidate Metabolite Assay.

Compound	Concentration Tested (µg/mL)
6-Acetyl morphine	10
Amitriptyline	100
Amphetamine	100
Chlorpromazine	50
Clomipramine	50
Cyclobenzaprine	10
Desipramine	50
Dextromethorphan	100
Doxepin	50
EDDP	100
EMDP	50

Compound	Concentration Tested (µg/mL)
Fentanyl	100
Fluoxetine	50
Imipramine	30
Ketamine	100
Meperidine	100
Methadone	100
Methapyrilene	10
Methylphenidate	25
Morphine	100
Morphine-3-glucuronide	50
Norcodeine	50
NorFentanyl	100
Norketamine	100
Normeperidine	100
Normorphine	50
Noroxycodone	50
Nortriptyline	25
Pentazocine (Talwin)	10
Phencyclidine	100
Risperidone	2
Thioridazine	50
Tramadol	100
Tramadol-N-Desmethyl	100
Tramadol-O-Desmethyl	100
Trazodone	10
Venlafaxine	100
Propyl 4-hydroxybenzoate	100
Methyl 4-hydroxybenzoate	100

Interference – Structurally Unrelated Compounds

High concentrations of the following structurally unrelated compounds were added into urine spiked with Methylphenidate Metabolite ($\pm 50\%$ of the cutoff concentration) and tested with the ARK Methylphenidate Metabolite Assay in both qualitative and semiquantitative modes. The substances listed below did not yield a false result relative to the 100 ng/mL cutoff.

Compound	Concentration Tested (µg/mL)	50 ng/mL (-50% Cutoff)	150 ng/mL (+50% Cutoff)
Acetaminophen	500	Negative	Positive
Acetylsalicylic acid	1000	Negative	Positive
Albuterol	100	Negative	Positive
Amobarbital	100	Negative	Positive
Benzoylcegonine	100	Negative	Positive
Buprenorphine	100	Negative	Positive
Buprenorphine glucuronide	10	Negative	Positive
Bupropion	50	Negative	Positive

Compound	Concentration Tested (µg/mL)	50 ng/mL (-50% Cutoff)	150 ng/mL (+50% Cutoff)
Caffeine	100	Negative	Positive
Carbamazepine	100	Negative	Positive
Codeine	100	Negative	Positive
Dihydrocodeine	100	Negative	Positive
Ecgonine	100	Negative	Positive
Ephedrine	100	Negative	Positive
Fluphenazine	25	Negative	Positive
Heroin	50	Negative	Positive
Hydrocodone	100	Negative	Positive
Hydromorphone	100	Negative	Positive
Ibuprofen	500	Negative	Positive
Levorphanol	50	Negative	Positive
Lidocaine	50	Negative	Positive
Maprotiline	50	Negative	Positive
Methaqualone	50	Negative	Positive
Metronidazole	300	Negative	Positive
Naloxone	50	Negative	Positive
Naltrexone	50	Negative	Positive
Nicotine	10	Negative	Positive
Norbuprenorphine	50	Negative	Positive
Oxazepam	100	Negative	Positive
Oxycodone	100	Negative	Positive
Oxymorphone	50	Negative	Positive
Phenobarbital	100	Negative	Positive
Propoxyphene	50	Negative	Positive
Ranitidine	100	Negative	Positive
Secobarbital	100	Negative	Positive
Tapentadol	50	Negative	Positive
Tilidine	50	Negative	Positive
Valproic acid	500	Negative	Positive

Interference – Endogenous Substances

High concentrations of the following endogenous substances were added into urine spiked with Methylphenidate Metabolite ($\pm 50\%$ of the cutoff concentration). No interference was observed when tested with the ARK Methylphenidate Metabolite Assay in both qualitative and semiquantitative modes.

Compound	Concentration Tested (mg/dL)	50 ng/mL (-50% Cutoff)	150 ng/mL (+50% Cutoff)
Acetone	1000	Negative	Positive
Ascorbic acid	200	Negative	Positive
Bilirubin (Conjugated)	2	Negative	Positive
Bilirubin (Unconjugated)	2	Negative	Positive
Creatinine	400	Negative	Positive
Ethanol	1000	Negative	Positive
Galactose	10	Negative	Positive
Glucose	3000	Negative	Positive
Hemoglobin	300	Negative	Positive
Human Albumin	500	Negative	Positive
Human Gamma Globulin	500	Negative	Positive
Oxalic Acid	30	Negative	Positive
Riboflavin	3.75	Negative	Positive
Sodium Chloride	900	Negative	Positive
Urea	1000	Negative	Positive

Interference – Boric Acid

One percent (1%) w/v of boric acid was added into urine spiked with Methylphenidate Metabolite ($\pm 50\%$ of the cutoff concentration) and tested with the ARK Methylphenidate Metabolite Assay in both qualitative and semiquantitative modes. Results are provided in the table below.

Compound	Concentration Tested	50 ng/mL (-50% Cutoff)	150 ng/mL (+50% Cutoff)
Boric Acid	1% w/v	Negative	Negative

Interference – Specific Gravity and pH

Urine samples with specific gravity values from 1.000 to 1.035 and pH values ranging from 3.0 to 11.0 were tested in the presence of the two levels of Methylphenidate Metabolite at $\pm 50\%$ of the cutoff concentration. No interference was observed when tested with the ARK Methylphenidate Metabolite Assay in both qualitative and semiquantitative modes.

Method Comparison

A total of one hundred nineteen (119) unaltered clinical urine specimens that are not individually identifiable were analyzed for Methylphenidate Metabolite with the ARK Methylphenidate Metabolite Assay in both qualitative and semiquantitative modes and the results were compared to LC-MS/MS. The LC-MS/MS confirmatory method was performed by a licensed reference laboratory. Results are summarized in the tables below.

		LC-MS/MS	
		(+)	(-)
ARK Methylphenidate Metabolite Assay (100 ng/mL Cutoff)	(+)	64	1*
	(-)	0	54

**Discordant Result*

Sample ID Number	ARK Qualitative Result	ARK Semiquantitative Result	LC-MS/MS Result
11P	Positive	123.7 ng/mL	94 ng/mL

12 References

1. Prescribing Information. 2017. Ritalin®. Novartis Pharmaceuticals Corporation (East Hanover, New Jersey).
2. United States Drug Enforcement Administration (DEA). Controlled Substances Act (CSA).
3. Morton, W.A & Stockton, G.G. 2000. Methylphenidate Abuse and Psychiatric Side Effects. *Primary Care Companion J Clin Psychiatry*. **2(5)**: 159-164.
4. Hungund, B.L. et al. 1979. Pharmacokinetics of Methylphenidate in Hyperkinetic Children. *Br. J. Clin. Pharmacol.* **8**: 571-576.
5. Wells, R. et al. 1974. Gas-Liquid Chromatographic Procedure for Measurement of Methylphenidate Hydrochloride and Its Metabolite, Ritalinic Acid, in Urine. *Clin. Chem.* **20(4)**: 440-443.
6. Department of Health and Human Services (DHHS), Substance Abuse and Mental Health Services Administration. Mandatory Guidelines for Federal Workplace Drug Testing Programs. Federal Register / Vol. 69, No. 71 / Tuesday, April 13, 2004 (Effective Date: November 1, 2004) / Notices.
7. Department of Health and Human Services (DHHS), Substance Abuse and Mental Health Services Administration. Mandatory Guidelines for Federal Workplace Drug Testing Programs. Federal Register / Vol. 82, No. 13 / Monday, January 23, 2017 (Effective Date: October 1, 2017) / Notices.

13 Trademarks

ARK™ is a trademark of ARK Diagnostics, Inc.

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