REF ARI-RGT

MyCare Psychiatry Total Aripiprazole Assay Kit

INDICATIONS FOR USE

The MyCare Psychiatry Total Aripiprazole Assay Kit is intended for the *in vitro* quantitative measurement of total aripiprazole (aripiprazole plus dehydroaripiprazole) in human serum using automated clinical chemistry analyzers. Measurements obtained are used for monitoring patient adherence to aripiprazole therapy to help ensure appropriate treatment.

SUMMARY AND EXPLANATION OF THE TEST

Aripiprazole (7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-di-hydrocarbostyril) is a quinolone derivative atypical anti-psychotic agent. It has partial agonistic activity at dopamine D2 receptors and serotonin 5-HT1A receptors and potent antagonistic activity on serotonin 5-HT2A receptors.[1,2] The oral medication is indicated for the treatment of schizophrenia, acute treatment of manic and mixed episodes associated with bipolar disorder, adjunctive treatment of major depressive disorder, irritability associated with autistic disorder, and Tourette's disorder. The injectable is indicated for agitation associated with schizophrenia or bipolar mania. The major metabolite of aripiprazole, dehydroaripiprazole, is also pharmaceutically active.[1] The therapeutic effect of aripiprazole is due to the total exposure to both aripiprazole and the active metabolite (dehydroaripiprazole).[3] The total aripiprazole assay measures the total active aripiprazole in patient serum: aripiprazole plus dehydroaripiprazole.

Nonadherence to medication is well known for patients with severe mental illness.[4] While adherence to medication is critical to successful treatment outcomes, adherence is also least likely to be accurately assessed.[5,6] Measurement of total aripiprazole provides clinicians with objective evidence of concentrations that may be related to patient adherence.[7]

The total aripiprazole assay is a homogenous two reagent nanoparticle agglutination assay used for detection of total aripiprazole in human serum. It is based on competition between drug and drug-conjugates for binding to drug specific antibodies covalently bound to nanoparticles. The extent of particle aggregation can be followed spectrophotometrically on clinical chemistry analyzers.

REAGENTS

MyCare Psychiatry Total Aripiprazole Assay Kit **REF** ARI-RGT	Quantity x Volume
Reagent 1 **R1** Reaction buffer that contains drug-conjugate, protein and buffer	1 x 10.0 mL
Reagent 2 **R2** Nanoparticle reagent that contains monoclonal antibody bound to nanoparticles in a buffered solution	1 x 5.0 mL

WARNINGS AND PRECAUTIONS

- For In Vitro Diagnostic Use Only.
- For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examination and other findings.
- Exercise normal precautions required for handling all laboratory reagents.
- Follow reagent handling instructions. Improper mixing of reagents can affect assay performance.
- All components of the total aripiprazole assay contain less than 0.1% sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Seek immediate medical attention if reagents are ingested or come into contact with eyes. When disposing of such reagents, always flush with large amounts of water to prevent accumulation of azide.

REAGENT HANDLING

The total aripiprazole assay reagents are ready to use. Mix the reagents (R1 and R2) by gently inverting five times, avoiding the formation of bubbles then place them on the analyzer.

Mix the reagents (R1 and R2) before pouring them into any analyzer-specific (secondary) reagent carrier. Before placing analyzer-specific (secondary) reagent carriers on the analyzer, mix the reagents (R1 and R2) by gently inverting five times, avoiding the formation of bubbles.

STORAGE AND STABILITY

Store reagents refrigerated at 2 - 8°C. Do not freeze.

When stored and handled as directed, unopened reagents are stable until the expiration date on the label. Improper storage of reagents can affect assay performance.

SPECIMEN COLLECTION AND HANDLING

Serum is required. Trough or C_{min} samples at steady state have been recommended for testing antipsychotics.[6] After two weeks of treatment on the same dose, collect samples before the next dose.[8] For long lasting injectables collect the sample before the next dose.[7]

Prepare serum within 3 days of blood collection. Blood and serum samples may be stored at room temperature or 2 - 8°C. Store serum for up to 7 days before measuring. Freeze (≤ -20°C) for longer storage. Ensure the sample is thawed and thoroughly mixed before measuring. Avoid repeated freezing and thawing of samples.

PROCEDURE

Materials Provided:

REF ARI-RGT – MyCare Psychiatry Total Aripiprazole Assay Kit

Materials Required – Provided Separately:

REF MCP2-CAL – MyCare Psychiatry Calibrator Kit 2

REF MCP2-CON - MyCare Psychiatry Control Kit 2

Instruments

Reagents may need to be transferred to analyzer-specific reagent containers.

The performance of applications not validated by Saladax Biomedical, Inc. is not warranted and must be user defined.

Assay

To run the assay, see the instrument specific application sheet and appropriate analyzer operator's manual.

Calibration

Perform a full calibration using the six calibrators in the Calibrator Kit 2. Verify the calibration by testing the low, medium, and high controls in the Control Kit 2.

Calibration Frequency - Calibration is recommended:

- After a reagent (kit) lot change,
- After performance of major instrument maintenance,
- As required following quality control procedures.

Quality Control (QC)

Each laboratory should establish its own QC procedures for the total aripiprazole assay kit. All quality control requirements and testing should be performed in accordance with local, state and/or federal regulations or accreditation requirements. Good laboratory practice suggests that at least two QC concentrations be tested each day patient samples are measured, and each time calibration is performed. Ensure that the quality control results meet the acceptance criteria before reporting patient results.

RESULTS

The concentration result is automatically calculated from the non-linear calibration curve by the analyzer. Report results in ng/mL or nmol/L. The conversion factor from ng/mL is 2.23 x ng/mL = 1 nmol/L.

LIMITATIONS OF THE PROCEDURE

The total aripiprazole assay has been validated for serum. Do not use serum separator tubes.

As with any assay utilizing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Samples containing such antibodies can potentially produce erroneous total aripiprazole results, which are inconsistent with the patient's clinical profile.

For samples containing 150 and 500 ng/mL total aripiprazole, 50 ng/mL of cariprazine caused assay biases of 164% and 71% respectively. Elevated levels of aripiprazole may be seen in patients administered cariprazine. For samples containing 150 and 500 ng/mL total aripiprazole, 42,000 ng/mL of lamotrigine (3X the therapeutic level) caused assay biases of 40%. Elevated levels of aripiprazole may be seen in patients administered lamotrigine.

EXPECTED VALUES

The therapeutic range for total aripiprazole in serum is not fully established. A therapeutic range from 150 to 500 ng/mL has been proposed for aripiprazole plus dehydroaripiprazole.[7] Measured concentrations for adherent patients at steady state are expected to be in the measuring range of the assay. Therapeutic drug monitoring of total aripiprazole has been recommended because of high interpatient variability, unpredictable response, and the importance of adherence for successful therapy.[7] The complexity of the clinical state, individual differences in sensitivity, and co-administered medications may contribute to different requirements for optimal total aripiprazole blood levels. Users should investigate the transferability of the expected values to their own patient population and if necessary determine their own reference range. For diagnostic purposes the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings. Clinicians should carefully monitor patients during therapy initiation and dose adjustments. It may be necessary to obtain multiple samples to determine expected variation of optimal (steady state) concentrations for individual patients.

SPECIFIC PERFORMANCE DATA

Typical performance data for the total aripiprazole assay obtained on a Beckman Coulter AU480 are shown below. Results obtained in individual laboratories may differ from these data.

Precision

Within-laboratory precision and repeatability were verified throughout the measuring range according to CLSI Guideline EP05-A3.[9] Three Control Kit 2 controls (Control 1, 2, 3), two serum pools spiked with both aripiprazole and dehydroaripiprazole to mimic the metabolite ratio found in clinical samples (Serum 1, 2), and two pools of clinical samples (Clinical 1, 2) were tested.

Sample	N	Mean (ng/mL)	Repeatability	Within-Laboratory
			CV	CV
Control 1	80	49	6.5%	8.3%
Control 2	80	198	2.3%	4.0%
Control 3	80	682	2.2%	3.9%
Serum 1	80	45	6.5%	9.5%
Serum 2	80	959	2.6%	4.3%
Clinical 1	80	150	3.5%	4.1%
Clinical 2	80	503	2.6%	4.1%

Limit of Quantitation (LoQ) and Limit of Detection (LoD)

The lower limits of quantitation and detection were established using CLSI guideline EP17-A2.[10]

LoQ

The LoQ was determined with an accuracy goal at the LoQ of ≤ 35% total error (Westgard model). The LoQ of the total aripiprazole assay is 45 ng/mL.

LoD

The LoD is the lowest amount of analyte that can be reliably detected (≥ 95% of results greater than the limit of blank.). The LoD of the total aripiprazole assay is 22 ng/mL.

Result Reporting

Each laboratory should determine reporting criteria for total aripiprazole concentrations. The following suggestion from CLSI EP17-A2 may be appropriate:[10]

Result ≤ LoB - report "not detected; concentration < LoD"

LoB < Result < LoQ - report "analyte detected; concentration < LoQ"

Result ≥ LoQ - report the result as measured

Measurement Range

The measurement range of the total aripiprazole assay is 45 – 1,000 ng/mL.

Specificity

Metabolism

Aripiprazole is metabolized in the liver by CYP3A4 and CYP2D6. The major metabolite dehydroaripiprazole also has pharmacological activity.[1,3] At steady state, its concentration is ~40% of the parent drug.[1] The other major metabolite, the acid product of N-dealkylation (OPC-3373) is also present in serum. Another minor metabolite (DCPP) is found at < 20% of the parent drug.

Specificity for the following metabolites was tested in the absence and presence of total aripiprazole at 150, 500, and 1,000 ng/mL.

Compound	Tested at (ng/mL)	% Bias
3,4-dihydro-7-(3'carboxy) propoxy-2(1H) quinolinone (OPC-3373)	475	3%
1-(2,3-dichlorophenyl) piperazine (DCPP)	50	6%

Interfering Substances

Testing of interferents was conducted according to CLSI guidelines for interference.[11-13] No significant assay bias was observed from samples with the following endogenous interferents at the given levels:

Interferent	Level	
Rheumatoid Factor	508 IU/mL	
Human Serum Albumin	10.8 g/dL	108 g/L
Human Immunoglobulin G	12.1 g/dL	121 g/L
Icteric Interference	43.5 mg/dL	744 µmol/L
Lipemic Interference	614 mg/dL	6.9 mmol/L
Hemolysate	1,050 mg/dL	

Cross-reactivity

Specificity for the following cross-reactants was tested in the absence and presence of total aripiprazole at 150, 500, and 1,000 ng/mL.

Cross-reactivity was tested according to CLSI guidelines for interferences.[11-13] The following compounds did not interfere with the total aripiprazole assay: assay bias was ≤ 13%.

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Acetaminophen	200,000	Acetazolamide	60,000
Acetylsalicylic acid	500,000	Albuterol	1,000
Alendronate sodium	1,000	Alpha - tocopherol	130,000
Alprazolam	2,000	Amantadine Hydrochloride	10,000
Amikacin sulfate	144,000	Amiloride HCl dihydrate	500
Amisulpride	1,200	Amitriptyline	1,000
Amlodipine besylate	100	S (+)-amphetamine	1,000
Amoxapine	2,900	Amoxicillin	80,000
L-ascorbic acid	60,000	Asenapine	500
Atomoxetine	7,900	Atorvastatin calcium	800
Baclofen	3,000	Benztropine	600
Betamethasone	400	Biotin	3,600
Biperiden	300	Blonanserin	100
Brexpiprazole	1,000	Bromperidol	100
Budesonide	50	Bupropion	3,000

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Buspirone	200	Caffeine	108,000
Calcium carbonate	315,000	Cannabidiol	100
Cannabinol	100	Carbamazepine	45,000
L-Carnosine	100,000	Cefalexin	200,000
Celecoxib	8,800	Cetirizine dihydrochloride	4,400
8-chloro-theophylline	3,000	Chlorpromazine HCl	3,300
Cimetidine	30,000	Ciprofloxacin	12,000
Citalopram HBr	5,500	Clindamycin	51,000
Clonazepam	300	Clotiapine	500
Clotrimazole	50	Clozapine	1,800
Codeine	2,000	Cortisol	300
(-)-cotinine	2,000	Cyclosporin A	9,000
Desloratadine	600	Desvenlafaxine	800
Dextromethorphan	1,000	Diazepam	30,000
Diphenhydramine HCl	6,000	Divalproex Sodium	400,000

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Docosahexaenoic acid ethyl ester	150,000	Donepezil	50,000
Doxycycline HCl	35,000	Droperidol	200
D-Serine	100,000	Duloxetine	200
Erythromycin	138,000	Escitalopram	200
Estradiol	10	Eszopiclone	200
Ethanol	10,000,000	Famotidine	2,500
Fenofibrate	50,000	Fentanyl	600
Fluoxetine HCl	4,000	Fluticasone propionate	50
Fluvoxamine	2,000	Folic acid	15
Furosemide	60,000	Galantamine	200
Gentamicin sulfate	30,000	Glyburide	2,000
Haloperidol	1,000	Heparin sodium salt	50 U/mL
Hydrochlorothiazide	6,000	Hyoscine (Scopolamine HBr)	100
Hyperforin (St. John's Wort)	200	Hypericin (St. John's Wort)	100
Ibuprofen	500,000	Iloperidone	100
Imipramine	700	Indinavir sulfate	400
Lactulose	10,000	Lamivudine	10,500
Lamotrigine	15,000	Lansoprazole	9,400
Levonorgestrel	100	Lisinopril dihydrate	350
Lithium carbonate	250,000	Lorazepam	1,000
Lovastatin	500	Loxapine	300
Lurasidone	400	Meclizine dihydrochloride	500
Metformin	40,000	Methotrimeprazine	600
Methylphenidate HCl	350	Metoclopramide HCl	500
Metoprolol tartrate	5,000	Metronidazole	123,000
Midazolam	3,800	Milnacipran	10,000
Mirtazapine	900	Mometasone furoate	50
Morphine	7,800	Naltrexone	200
Naproxen sodium	500,000	Nateglinide	30,000
Nefazodone HCl	6,000	Nicotine	1,000
Nicotinic acid	27,900	Nordiazepam	5,000
Nortriptyline	1,200	Olanzapine	300
Omeprazole	8,400	Oxazepam	5,000
Oxcarbazepine	105,000	Oxycodone	500
Paliperidone	60	Pantothenic acid	1,800
Paroxetine	1,200	Penicillin V	42,000
Perazine	1,400	Perlapine	150
Perphenazine	100	Phenobarbital	690,000
Phentermine	500	Phenytoin	60,000
Pimozide	100	Pipamperone dihydrochloride	1,200
Potassium EDTA	1000	Pravastatin sodium	300
Prednisolone	3,000	Pregabalin	22,500
Procyclidine	1,900	Promethazine	1,200
R,R (-)-pseudoephedrine	10,000	S,S (+)-pseudoephedrine	10,000
Pyridoxine HCl	100	Quetiapine	2,800
Quinidine	15,000	Raloxifene	50
Ranitidine	10,500	Retinol	4,000
Riboflavin	200	Rifampicin	65,000
Risperidone	200	Rosuvastatin calcium	200
Salicylic acid	500,000	Sarcosine	1,500
Sertindole	300	Sertraline hydrochloride	1,000
Simvastatin	1,700	Sodium benzoate	400,000
Sodium fluoride	900	Spironolactone	600
Sulfamethoxazole	400,000	Sulpiride	50,000
Temazepam	5,000	Terbinafine	9,000
Theophylline	60,000	Thiamine HCl	500
Topiramate	75,000	Trazodone HCl	14,000
Triamcinolone acetonide	300	Triamterene	9,000
Triazolam	40	Valproic acid	500,000
Vancomycin HCl	120,000	Varenicline	50
Venlafaxine HCl	700	Vitamin B12	50
Vitamin D2	200	Vitamin K1	50
Warfarin	75,000	Ziprasidone	600
Zolpidem hemitartrate	5,000	Zonisamide	120,000
Zopiclone	200	Zuclopenthixol	300

Recovery

The recovery of total aripiprazole was assessed in the 3 controls, two spiked serum pools and two clinical pools measured for the EP05-A3 precision performance study. The percent recovery was determined by dividing the mean measured concentration of each sample by the expected concentration total aripiprazole. All mean recoveries were within 88% to 114%.

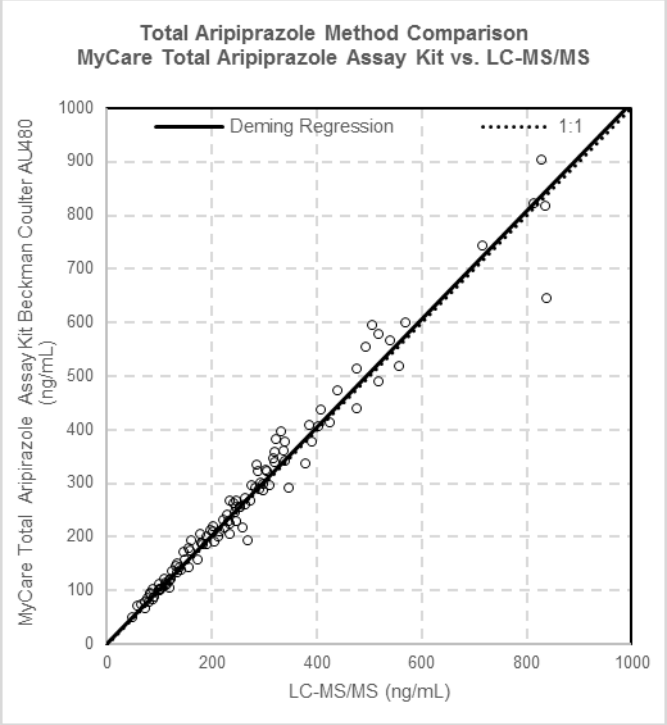
Linearity

The linearity of the total aripiprazole assay was verified according to CLSI guideline EP6-A.[14] Eleven linearity samples covering the measuring range were prepared in human serum spiked with aripiprazole. Deviation from linearity (n=5) was ≤ 10%. The assay was linear across the measuring range from 45 – 1,000 ng/mL.

Method Comparison

Results of the total aripiprazole assay were compared to a validated LC-MS/MS according to CLSI guideline EP09-A3.[15] Deming regression analysis was performed with 110 patient samples. Results are shown for one lot.

Deming Regression Statistics Total Aripiprazole Assay vs. LC-MS/MS	
Slope	1.01
Intercept	2.56
Correlation Coefficient (R)	0.98
N	110
Concentration Range (LC-MS/MS)	48 - 839



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SYMBOLS USED

IVD	*in vitro* Diagnostic Device		Consult Instructions for Use
REF	Catalog Number		Use By
LOT	Batch Code		Temperature Limitation
	Manufacturer	**Rx only**	For Prescription Use Only
R1 R2	Reagent 1 Reagent 2	(N) x	Gently invert reagents (R1 and R2) N number of times prior to use
CE	CE mark	EC REP	Authorized Representative in the European Community

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