REF OLZ-RGT

MyCare Psychiatry Olanzapine Assay Kit

INDICATIONS FOR USE

The MyCare Psychiatry Olanzapine Assay Kit is intended for the *in vitro* quantitative measurement of olanzapine in human serum using automated clinical chemistry analyzers. Measurements obtained are used for monitoring patient adherence to olanzapine therapy to help ensure appropriate treatment.

SUMMARY AND EXPLANATION OF THE TEST

Olanzapine (2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-b] [1,5]benzodiazepine) is an atypical antipsychotic in the thienobenzodiazepine class.[1] It is a serotonin and dopamine receptor antagonist with anticholinergic properties indicated for the treatment of schizophrenia and acute treatment of manic or mixed episodes associated with bipolar I disorder (given alone or as an adjunct to valproate or lithium),[1] while an injectable form is indicated for treatment of acute agitation associated with schizophrenia and bipolar I mania.[2] Used in conjunction with fluoxetine, olanzapine is used for the treatment of depressive episodes associated with bipolar I disorder and also for treatment resistant depression.[1]

Nonadherence to medication is well known for patients with severe mental illness.[3] While adherence to medication is critical to successful treatment outcomes, adherence is also least likely to be accurately assessed.[4,5] Measurement of olanzapine provides clinicians with objective evidence of concentrations that may be related to patient adherence.[6]

The olanzapine assay is a homogenous two reagent nanoparticle agglutination assay used for detection of olanzapine 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 Olanzapine Assay Kit **REF** OLZ-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 olanzapine 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 olanzapine 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 analyser, 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. Olanzapine is taken in the evening or at bedtime, making a twelve-hour concentration a practical option, one that has been used in multiple studies.[6-8] Olanzapine reaches steady state after 7 days on the same dose.[1] For long lasting injectables collect the sample before the next dose.[6]

Prepare serum from whole blood at room temperature within 8 hours of blood collection. If whole blood is stored at 2 - 8°C, prepare serum within 3 days. Serum samples may be stored at room temperature or 2 - 8°C. Serum may be stored 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 OLZ-RGT – MyCare Psychiatry Olanzapine 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 five calibrators CAL A, B, C, D and E from the Calibrator Kit 2. Verify the calibration by testing the low and medium controls from 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 olanzapine assay kit. All quality control 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.

Specimen Dilution Procedure

Samples containing olanzapine in concentrations greater than 114 ng/mL can be diluted 1:2 (1-part sample plus two parts water) to give an upper range of 342 ng/mL. Refer to the instrument specific operation manual for an automatic dilution protocol (by cuvette only) of

olanzapine samples with water. Alternatively, specimens out of range can be manually diluted 1:2 with deionized water and placed in the sample rack for analysis.

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 3.20 x ng/mL = nmol/L.

LIMITATIONS OF THE PROCEDURE

The olanzapine 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 olanzapine results, which are inconsistent with the patient's clinical profile.

For samples containing 20 ng/mL olanzapine, the addition of asenapine (500 ng/mL) or donepezil (50,000 ng/mL) caused assay biases ≥ 35%. Elevated levels of olanzapine may be seen in patients administered asenapine or donepezil.

Elevated levels of olanzapine may be seen in patients co-administered clozapine. Patients taking clozapine should not be tested with the MyCare Olanzapine Assay Kit.

EXPECTED VALUES

The therapeutic range for olanzapine in serum is not fully established. A therapeutic range from 20 to 80 ng/mL has been proposed for olanzapine.[6] Measured concentrations for adherent patients at steady state are expected to be in the measuring range of the assay. Therapeutic drug monitoring of olanzapine has been recommended because of high interpatient variability, unpredictable response, and the importance of adherence for successful therapy.[6] The complexity of the clinical state, individual differences in sensitivity, and co-administered medications may contribute to different requirements for optimal olanzapine 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 olanzapine 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] Two Control Kit 2 controls and two olanzapine spiked pools (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	3.1%	4.6%
Control 2	80	106	1.7%	1.9%
Serum 1	80	48	2.9%	3.7%
Serum 2	80	101	1.5%	2.4%
Clinical 1	80	20	5.6%	9.0%
Clinical 2	80	76	2.4%	3.7%

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 olanzapine assay is 22 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 olanzapine assay is 18 ng/mL.

Result Reporting

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

Result < LoD - report “not detected; concentration < LoD”

LoD < Result < LoQ - report “analyte detected; concentration < LoQ”

Result ≥ LoQ - report the result as measured

Measurement Range

The measurement range of the olanzapine assay is 22 – 114 ng/mL.

Specificity

Metabolism

Olanzapine is extensively metabolized in the liver. The major metabolites N-desmethyl-olanzapine and N-glucuronide are inactive at circulating concentrations and occur at lower concentrations than the parent compound,[11] as do the minor metabolites olanzapine N-oxide and 2-hydroxymethyl olanzapine.[12] When the following metabolites were tested with 80 ng/mL olanzapine the assay bias was ≤ 18%. This should not introduce a clinically relevant bias given the low concentration of these minor metabolites.[11]

Specificity for the following metabolites was tested in the absence and presence of olanzapine at 20, 80, and 100 ng/mL

Compound	Tested at (ng/mL)	% Bias
N-desmethyl-olanzapine	50	4%
Olanzapine N-oxide	50	18%
2-hydroxymethyl olanzapine	50	4%

Interfering Substances

Testing of interferents was conducted according to CLSI guidelines for interferences.[13-15] 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	13.4 g/dL	134 g/L
Human Immunoglobulin G	12.2 g/dL	122 g/L
Icteric Interference	44.9 mg/dL	767 µmol/L
Lipemic Interference	1,760 mg/dL	19.9 mmol/L
Hemolysate	1,050 mg/dL	

Cross-reactivity

Specificity for the following cross-reactants was tested in the absence and presence of olanzapine at 20, 80, and 100 ng/mL.

Cross-reactivity was tested according to CLSI guidelines for interference.[13-15] The following compounds did not interfere with the olanzapine assay: assay bias was ≤ 27% at 20 ng/mL of olanzapine and ≤ 7% at 80 and 100 ng/mL olanzapine.

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

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Amikacin sulfate	144,000	Amiloride HCl dihydrate	500
Amisulpride	1,200	Amitriptyline	1,000
Amlodipine besylate	100	Amoxapine	2,900
Amoxicillin	80,000	S (+)-amphetamine	1,000

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Aripiprazole	1,400	L-ascorbic acid	60,000
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
Buspirone	200	Caffeine	108,000
Calcium carbonate	315,000	Cannabidiol	100
Cannabinol	100	Carbamazepine	45,000
Cariprazine	50	L-Carnosine	100,000
Cefalexin	200,000	Celecoxib	8,800
Cetirizine dihydrochloride	4,400	8-chlorotheophylline	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
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
Docosahexaenoic acid ethyl ester	150,000	Doxycycline HCl	35,000
Droperidol	200	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	42,000	Lansoprazole	9,400
Levonorgestrel	100	Lisinopril dihydrate	350
Lithium carbonate	250,000	Lorazepam	1,000

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
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	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	1,000
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	D-Serine	100,000
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	14,700
Triamcinolone acetonide	300	Triamterene	9,000
Triazolam	40	Valproic acid	500,000
Vancomycin HCl	120,000	Varenicline	50
Venlafaxine HCl	700	Vitamin B12	50

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Vitamin D2	200	Vitamin K1	50
Warfarin	75,000	Ziprasidone	600

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Zolpidem hemitartrate	5,000	Zopiclone	200
Zonisamide	120,000	Zuclopenthixol	300

Recovery

The recovery of olanzapine was assessed for the 2 controls and two spiked serum 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 of added olanzapine. The percent recovery ranged from 90 to 105%.

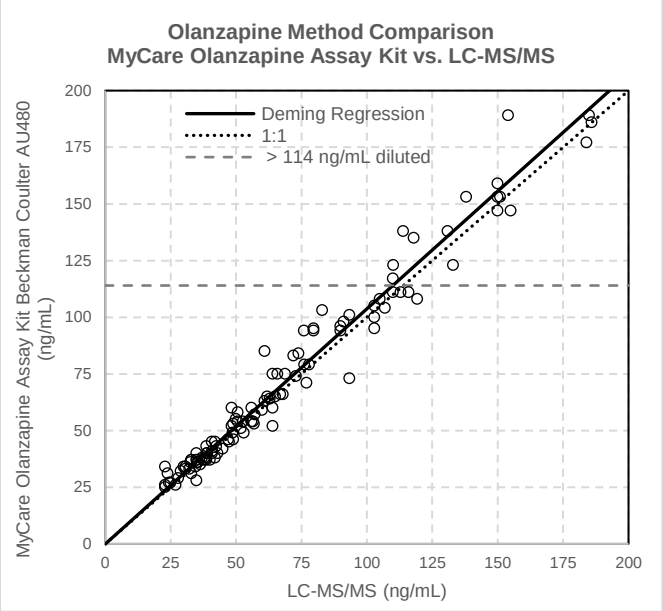
Linearity

The linearity of the olanzapine assay was verified according to CLSI guideline EP6-A.[16] Eleven linearity samples covering the measuring range were prepared in human serum spiked with olanzapine. The assay was linear across the measuring range from 22 – 114 ng/mL. Deviation from linearity (n=5) was ≤ 5% in the measuring range.

Method Comparison

Results of the olanzapine assay were compared to a validated LC-MS/MS according to CLSI guideline EP09-A3.[17] Deming regression analysis was performed with 113 patient samples. Patient samples above the test range of the olanzapine assay kit were diluted as described under Specimen Dilution Procedure. Results are shown for one lot.

Deming Regression Statistics Olanzapine Assay vs. LC-MS/MS	
Slope	1.038
Intercept	-0.1
Correlation Coefficient (R)	0.98
N	113
Concentration Range (LC-MS/MS)	23 - 186



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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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