REF CLZ-RGT

MyCare Psychiatry Clozapine Assay Kit

INDICATIONS FOR USE

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

SUMMARY AND EXPLANATION OF THE TEST

Clozapine 8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo [b,e] [1,4] diazepine is a tricyclic dibenzodiazepine derivative atypical antipsychotic agent used for treatment resistant schizophrenia and reducing suicidal behavior in schizophrenia and schizoaffective disorder.[1]

Nonadherence to medication is well known for patients with severe mental illness.[2] While adherence to medication is critical to successful treatment outcomes, adherence is also least likely to be accurately assessed.[3,4] Measurement of clozapine provides clinicians with objective evidence of concentrations that may be related to patient adherence.[5]

The clozapine assay (US Patent 8,771,972) is a homogeneous two reagent nanoparticle agglutination assay used for detection of clozapine in human serum and plasma. 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 analysers.

REAGENTS

MyCare Psychiatry Clozapine Assay Kit **REF** CLZ-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 clozapine 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 clozapine 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 analyser.

Mix the reagents (R1 and R2) before pouring them into any analyser-specific (secondary) reagent carrier. Before placing analyser-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 or EDTA plasma is required. Trough or C_{min} samples at steady state have been recommended for testing antipsychotics.[5] After one week of treatment on the same dose, collect samples before the next dose.[6]

Prepare serum or plasma within 3 days of blood collection. Blood, serum and plasma samples may be stored at room temperature or 2 - 8°C. Store serum and plasma 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 CLZ-RGT – MyCare Psychiatry Clozapine 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 analyser-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 analyser 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 clozapine 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 analyser. Report results in ng/mL or nmol/L. The conversion factor from ng/mL clozapine is 3.06 x ng/mL = 1 nmol/L.

LIMITATIONS OF THE PROCEDURE

The clozapine assay has been validated for serum and plasma. Do not use serum or plasma 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 clozapine results, which are inconsistent with the patient's clinical profile.

EXPECTED VALUES

The therapeutic range for clozapine in serum and plasma is not fully established. A therapeutic range from 350 to 600 ng/mL[5] has been proposed. Measured concentrations for adherent patients at steady state are expected to be in the measuring range of the assay. Therapeutic drug monitoring of clozapine has been recommended because of high interpatient variability, unpredictable response, and the importance of adherence for successful therapy.[5] The complexity of the clinical state, individual differences in sensitivity, and co-administered medications may contribute to different requirements for optimal clozapine 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 clozapine 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.[7] Three Control Kit 2 controls, and four pools of clinical samples (Clinical 1, 2, 3, 4) were tested.

Sample	N	Mean (ng/mL)	Repeatability	Within-Laboratory
			CV	CV
Control 1	80	156	3.6%	5.7%
Control 2	80	474	2.4%	4.8%
Control 3	80	945	2.9%	5.2%
Clinical 1	80	148	3.6%	6.6%
Clinical 2	80	338	2.2%	4.2%
Clinical 3	80	577	2.6%	4.3%
Clinical 4	80	926	3.6%	5.1%

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

The lower limits of quantitation and detection were established using CLSI guideline EP17-A2.[8]

LoQ

The LoQ was determined with an accuracy goal at the LoQ of ≤ 35% total error (Westgard model). The LoQ of the clozapine assay is 68 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 clozapine assay is 39 ng/mL.

Result Reporting

Each laboratory should determine reporting criteria for clozapine concentrations. The following suggestion from CLSI EP17-A2 may be appropriate:[8]

- 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 clozapine assay is 68 – 1,500 ng/mL.

Specificity

Metabolism

Clozapine is extensively metabolized in the liver by CYP1A2 and to a lesser extent by CYP2D6 and CYP3A4. There are two major metabolites in blood: norclozapine and clozapine N-oxide, which have limited and no activity respectively.[1]

Specificity for the following metabolites was tested in the absence and presence of clozapine at 350 and 600 ng/mL.

Compound	Tested at (ng/mL)	% Bias
Clozapine N-oxide	250	2%
8-Hydroxy-8-deschloro-clozapine	100	9%
Norclozapine	800	2%

Interfering Substances

Testing of interferents was conducted according to CLSI guidelines for interference.[9-11] 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	10.9 g/dL	109 g/L
Human Immunoglobulin G	12.5 g/dL	125 g/L
Icteric Interference	18.18 mg/dL	310.88 µmol/L
Lipemic Interference	2,586 mg/dL	29 mmol/L
Hemolysate	1,050 mg/dL	

Cross-reactivity

Specificity for the following cross-reactants was tested in the absence and presence of clozapine at 350 and 600 ng/mL.

Cross-reactivity was tested according to CLSI guidelines for interference.[9-11] The following compounds had less than clinically relevant inferences (i.e., less than 10% bias in the clozapine assay).

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
Aripiprazole	1,400	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	Buspirone	200
Caffeine	108,000	Calcium carbonate	315,000

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
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	Dextro-methorphan	1,000
Diazepam	30,000	Diphenhydramine HCl	6,000
Divalproex Sodium	400,000	Docosahexaenoic acid ethyl ester	150,000
Donepezil	50,000	Doxycycline HCl	35,000
Droperidol	200	D-Serine	100,000
Duloxetine	200	Erythromycin	138,000

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Escitalopram	200	Estradiol	10
Eszopiclone	300	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	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	400	Omeprazole	8,400
Oxazepam	5,000	Oxcarbazepine	105,000
Oxycodone	500	Paliperidone	60

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
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	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	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 clozapine was assessed in the 3 controls, and 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 of clozapine. The percent recovery ranged from 97 to 116%.

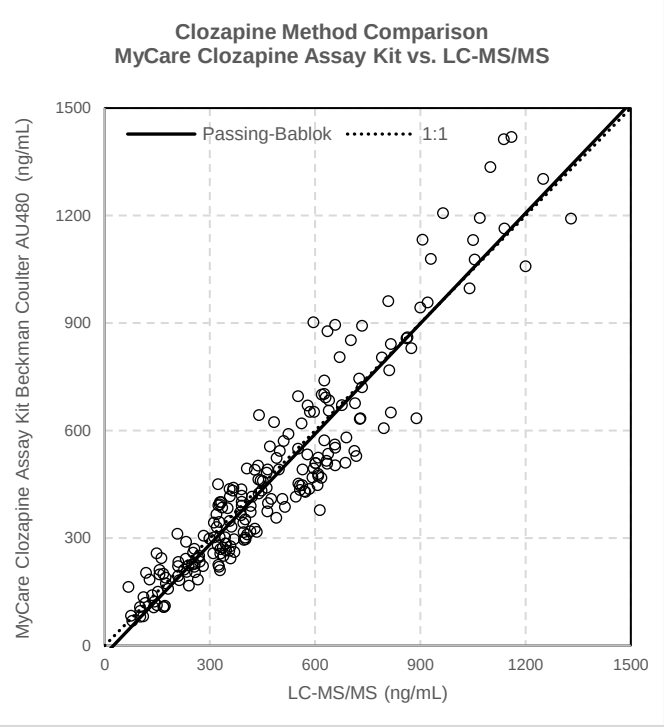
Linearity

The linearity of the clozapine assay was verified according to CLSI guideline EP6-A.[12] Eleven linearity samples covering the measuring range were prepared in human serum spiked with clozapine. Deviation from linearity (n=5) was ≤ 10%. The assay was linear across the measuring range from 68 to 1,500 ng/mL.

Method Comparison

Results of the clozapine assay were compared to a validated LC-MS/MS according to CLSI guideline EP09-A3.[13] Passing-Bablok regression analysis was performed with 213 patient samples.

Regression Statistics Clozapine Assay Kit vs. LC-MS/MS	
Slope	1.027
Intercept	-25.5
Correlation Coefficient (R)	0.9397
N	213
Concentration Range (LC-MS/MS)	68 - 1330



References

1. Novartis Pharmaceuticals Corporation. Clozaril (clozapine) prescribing information. 2015
2. Velligan DI, Weiden PJ, Sajatovic M, et al. Assessment of adherence problems in patients with serious and persistent mental illness: recommendations from the Expert Consensus Guidelines. J Psychiatr Pract. 2010;16(1):34-45.
3. Higashi K, Medic G, Littlewood KJ, Diez T, Granstrom O, De Hert M. Medication adherence in schizophrenia: factors influencing adherence and consequences of nonadherence, a systematic literature review. Ther Adv Psychopharmacol. 2013;3(4):200-218.
4. Haddad PM, Brain C, Scott J. Nonadherence with antipsychotic medication in schizophrenia: challenges and management strategies. Patient Relat Outcome Meas. 2014;5:43-62.
5. Hiemke C, Bergemann N, Clement HW, et al. Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017. Pharmacopsychiatry. 2018:51:9-62.
6. Grundmann M, Kacirova I, Urinovska R. Therapeutic drug monitoring of atypical antipsychotic drugs. *Acta Pharm.* 2014;64(4):387-401.
7. CLSI. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2014.
8. CLSI. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
9. CLSI. Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition CLSI document EP7-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
10. CLSI. Interference Testing in Clinical Chemistry. 3rd ed. CLSI guideline EP07. Wayne PA: Clinical and Laboratory Standards Institute; 2018.
11. CLSI. Supplemental Tables for Interference Testing in Clinical Chemistry. 1st ed. CLSI supplement EP37. Wayne PA: Clinical and Laboratory Standards Institute; 2018
12. NCCLS. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. NCCLS document EP6-A. Wayne, PA: NCCLS; 2003.
13. CLSI. Measurement Procedure and Bias Estimation Using Patient Samples; Approved Guideline-Third Edition. CLSI document EP09-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.

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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