REF CLZ-RGT

MyCare Psychiatry Clozapine Assay Kit

INTENDED USE

Rx only

The MyCare Psychiatry Clozapine Assay Kit is intended for the in vitro quantitative measurement of clozapine in adult human serum using automated clinical chemistry analyzers. Measurements obtained can be used to aid in the management of individuals prescribed clozapine for treatment-resistant schizophrenia. This assay should be used in conjunction with other clinical and laboratory findings and results from this test alone should not be used to make treatment decisions.

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]

It is useful to measure clozapine blood levels during dose titration.[2]

Measuring clozapine blood levels can also be useful when there are questions of medication adherence, efficacy, toxicity, medication interactions, or concern over other factors that may influence clozapine levels.[2]

The clozapine assay (US Patent 8,771,972) is a homogenous two reagent nanoparticle agglutination assay used for detection of clozapine 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

The kit contains sufficient reagent for 100 tests.

MyCare Clozapine Assay Kit REF CLZ-RGT	Quantity x Volume
Reagent 1 R1 Reaction buffer that contains drug-conjugate and protein in a buffered solution	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.
- 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.

Before use, mix the reagents by gently inverting three to five times, avoiding the formation of bubbles.

Mix the reagents before pouring them into any analyser-specific (secondary) reagent carrier. Before placing analyser-specific (secondary) reagent carriers on the analyser, mix the reagents by gently inverting three to 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 samples (12 hours after the last dose) at steady state (3 days after a dose change) are recommended for testing clozapine.[2]

Prepare serum within 2 hours of blood collection. Serum samples may be stored at room temperature or 2 to 8°C for up to 7 days before testing. Freeze (-70 to -90°C) serum for longer storage up to 10 months.

PROCEDURE

Assay

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

Instruments

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

Materials Provided:

REF CLZ-RGT – MyCare Clozapine Assay Kit

Materials Required – Provided Separately:

REF MCP2-CAL-US – MyCare Psychiatry Calibrator Kit 2

REF MCP2-CON-US - MyCare Psychiatry Control Kit 2

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 calibrator or 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 analyzer. Report results in ng/mL or nmol/L. The conversion factor from ng/mL clozapine is 3.06 x ng/mL = 1 nmol/L.

This assay should only be used in conjunction with other clinical and laboratory findings and results from this test alone should not be used to make treatment decisions.

Consider obtaining assay results before patient consultation.

If assay results are not yet available, treatment decisions should be based upon best clinical judgment at the time the patient is evaluated based on other clinical and laboratory findings.

LIMITATIONS OF THE PROCEDURE

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

EXPECTED VALUES

There is no definitive clozapine level that is associated with efficacy or toxicity. Efficacy for the majority of patients will be highest at levels above 350 ng/mL. For some other patients, however, clozapine levels as low as 200 ng/mL will provide response or prevention of relapse.[2]

As for any laboratory test, the clinical context should be taken into consideration when interpreting clozapine result. For example, assess dose related side effects and clinical evidence of toxicity if a clozapine level is higher than expected. Then if that patient's clinical status is not consistent with toxicity, determine the timing of the blood sample (e.g. peak vs. trough) and identify any potential drug interactions, changes in smoking status or incorrect specimen identification. Poor adherence, drug interactions or changes in smoking status may be relevant when clozapine levels are much lower than expected.

Compare assay results to results expected based on previous measurements, subject's baseline, suggested therapeutic range or values expected for the individual.

SPECIFIC PERFORMANCE DATA

Typical performance data for the clozapine assay obtained on a Beckman Coulter® AU480 are shown below.

Precision

Total precision and within-run precision were verified throughout the measuring range according to CLSI Guideline EP05-A3.[3] Three Control Kit 2 controls, four pools of clinical samples (Clinical 1, 2, 3, 4) and two serum pools spiked with clozapine (Spiked Serum 1, 2) were tested.

Sample	N	Mean (ng/mL)	Within - Run	Total
			CV	CV
Control 1	480	156	4.5%	5.4%
Control 2	480	474	3.7%	4.4%
Control 3	480	945	3.8%	4.5%
Clinical 1	480	148	4.6%	5.6%
Clinical 2	480	338	3.3%	4.0%
Clinical 3	480	577	3.3%	4.0%
Clinical 4	480	926	3.5%	4.8%
Spiked Serum 1	480	98	6.0%	7.8%
Spiked Serum 2	480	1,094	4.4%	6.6%

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

The lower limits of quantitation and detection were established using CLSI guideline EP17-A2.[4]

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.

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 serum: norclozapine and clozapine-N-oxide, which have limited and no activity respectively.[1]

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

Clozapine metabolites

Compound	Tested at (ng/mL)	% Cross-reactivity
Clozapine-N-oxide	250	3%
8-Hydroxy-8-deschloro-clozapine	100	9%
Norclozapine	2,700	1%

Interfering Substances

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	2586 mg/dL	29 mmol/L
Hemolysate	1050 mg/dL	

Cross-reactivity

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

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
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

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
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
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

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
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
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		

Linearity

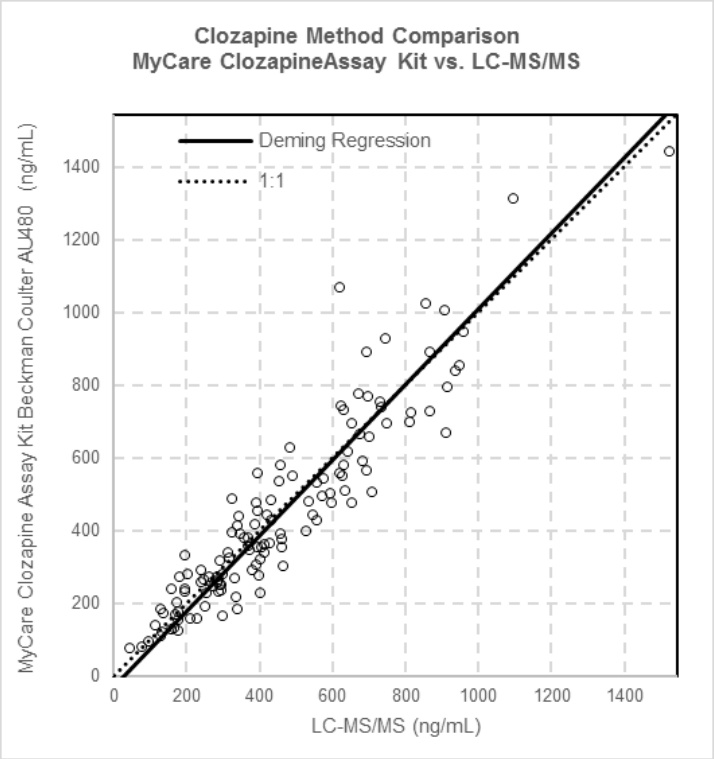
The linearity of the clozapine assay was verified according to CLSI guideline EP6-A.[5] Eleven linearity samples covering the measuring range were prepared in human serum spiked with clozapine. Linear regression gave a slope of 0.920 (CI 95%: 0.910 – 0.930) and an

intercept of 4.1 (CI 95%: 1.4 - 6.8) with an R = 0.9985. 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.[6] Deming regression analysis was performed with 123 patient samples.

Deming Regression Statistic	Result
Slope (99% confidence interval)	1.037 (0.939 to 1.135)
Y-intercept (99% confidence interval)	-27.8 (-78.3 to +22.7)
Correlation coefficient	0.9269
Average Bias (Average % Bias)	-10.9 ng/mL (- 2.4%)



Accuracy Performance Summary:

	Difference Range Between the Serum Clozapine Level by LC/MS-MS and the MyCare Psychiatry Clozapine Assay				
Concentration of Clozapine (ng/mL)	**Within 10% (n/N)**	**Within 15% (n/N)**	**Within 20% (n/N)**	**Within 30% (n/N)**	**Within 40% (n/N)**
68-350	30% (15/50)	42% (21/50)	60% (30/50)	78% (39/50)	84% (42/50)
350-1000	34% (24/71)	56% (40/71)	75% (53/71)	93% (66/71)	96% (68/71)
1000-1500	50% (1/2)	50% (1/2)	100% (2/2)	N/A	N/A

SYMBOLS USED

IVD	*in vitro* Diagnostic Device		Consult Instructions for Use
REF	Catalog Number		Use By
LOT	Batch Code		Temperature Limitation
	Manufacture	**Rx only**	For Prescription Use Only
R1 R2	Reagent 1 Reagent 2		

References

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2. The American Psychiatric Association Practice Guideline For The Treatment Of Patients With Schizophrenia. 3rd ed. [ebook] Washington, DC: American Psychiatric Association Publishing., p.90. Available at: https://psychiatryonline.org/doi/book/10.1176/appi.books.97808904248 [Accessed 8 September 2020].
3. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2014.
4. 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.
5. NCCLS. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. NCCLS document EP6-A. Wayne, PA: NCCLS; 2003.
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