Psychiatry Clozapine Assay Kit

INTENDED USE

Rx only

The Psychiatry Clozapine Assay Kit is intended for the *in vitro* quantitative measurement of clozapine in human serum and plasma using automated clinical chemistry analyzers. 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 analyzers.

REAGENTS

The kit contains sufficient reagent for 100 tests.

Psychiatry Clozapine Assay Kit **REF** C82914	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.
- The Safety Data Sheet (SDS) is available at https://www.saladax.com/bci_applications/

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 analyzer.

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. Do not use serum or plasma separator tubes.

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. Avoid repeated freezing and thawing of samples.

PROCEDURE

Assay

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

Materials Provided:

REF C82914 – Psychiatry Clozapine Assay Kit

Materials Required – Provided Separately:

REF C82911 – Psychiatry Calibrator Kit 2

REF C82912 – 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 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 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.

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

Clozapine metabolites

Compound	Tested at (ng/mL)	% Bias
Clozapine N-oxide	250	2%
Norclozapine	800	2%

Interfering Substances

Testing of interferents was conducted according to CLSI guideline EP7-A2.[9] No significant assay bias was observed from samples with the following endogenous interferents at the given levels:

Interferent	Level	
Rheumatoid Factor	508 IU/mL	
Total Protein Matrix Effect	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

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	40,000
Alprazolam	2,000	Amantadine Hydrochloride	10,000
Amikacin sulfate	100,000	Amiloride HCl dihydrate	500
Amisulpride	400	Amitriptyline	1,000
Amlodipine besylate	100	S (+)-amphetamine	1,000
Amoxapine	2,900	Amoxicillin	80,000
Aripiprazole	500	L-ascorbic acid	60,000
Asenapine	500	Atomoxetine	5,000
Atorvastatin calcium	600	Baclofen	3,000
Benztropine	400	Betamethasone	100
Biotin	300	Biperiden	100
Blonanserin	100	Brexpiprazole	1,000
Bromperidol	100	Budesonide	50

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Bupropion	3,000	Buspirone	200
Caffeine	60,000	Calcium carbonate	300,000
Cannabidiol	100	Cannabinol	100
Carbamazepine	30,000	Cariprazine	50
L-Carnosine	50,000	Cefalexin	200,000
Celecoxib	1,000	Cetirizine dihydrochloride	3,500
8-chlorotheophylline	3,000	Chlorpromazine HCl	2,500
Cimetidine	20,000	Ciprofloxacin	10,000
Citalopram HBr	750	Clindamycin	50,000
Clonazepam	150	Clotiapine	500
Clotrimazole	50	Codeine	2,000
Cortisol	300	(-)-Cotinine	2,000
Cyclosporin A	9,000	Desloratadine	600
Desvenlafaxine	400	Dextromethorphan	1,000
Diazepam	6,000	Diphenhydramine HCl	6,000

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Divalproex Sodium	50,000	Docosahexaenoic acid ethyl ester	150,000
Donepezil	50,000	Doxycycline HCl	35,000
Droperidol	100	D-Serine	100,000
Duloxetine	200	Erythromycin	60,000
Escitalopram	100	Eszopiclone	200
Ethanol	10,000,000	Famotidine	600
Fenofibrate	50,000	Fentanyl	600
Fluoxetine HCl	4,000	Fluticasone propionate	1
Fluvoxamine	2,000	Folic acid	15
Furosemide	60,000	Galantamine	100
Gentamycin sulfate	30,000	Glyburide	2,000
Haloperidol	1,000	Heparin sodium salt	50 U/mL
Hydrochlorothiazide	6,000	Hyoscine (Scopolamine HBr)	100
Ibuprofen	500,000	Iloperidone	10
Imipramine	700	Indinavir sulfate	400
Lactulose	10,000	Lamivudine	2000
Lamotrigine	15,000	Lansoprazole	1,000
Lisinopril dihydrate	350	Lithium carbonate	250,000
Lorazepam	1,000	Lovastatin	500
Loxapine	150	Lurasidone	100
Meclizine dihydrochloride	500	Metformin	40,000
Methotrimeprazine	200	Methylphenidate HCl	350
Metoclopramide HCl	500	Metoprolol tartrate	5,000
Metronidazole	120,000	Midazolam	1,000
Milnacipran	10,000	Mirtazapine	300
Mometasone furoate	50	Morphine	500
Naltrexone	50	Naproxen sodium	500,000
Nateglinide	20,000	Nefazodone HCl	3,500
Nicotinic acid	20,000	Nordiazepam	5,000
Nortriptyline	1,000	Olanzapine	300
Omeprazole	6,000	Oxazepam	5,000
Oxcarbazepine	35,000	Oxycodone	500

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Paliperidone	60	Pantothenic acid	150
Paroxetine	1,000	Penicillin V	6,000
Perazine	1,000	Perlapine	150
Perphenazine	100	Phenobarbital	50,000
Phentermine	500	Phenytoin	50,000
Pimozide	20	Pipamperone dihydrochloride	400
Potassium EDTA	1000	Pravastatin sodium	150
Prednisolone	3,000	Pregabalin	5,000
Procyclidine	1,000	Promethazine	1,200
R,R-(-)-pseudoephedrine	10,000	S,S-(+)-pseudo-ephedrine	10,000
Pyridoxine HCl	100	Quetiapine	500
Quinidine	12,000	Raloxifene	50
Ranitidine	6,000	Retinol	4,000
Riboflavin	200	Rifampicin	65,000
Risperidone	60	Rosuvastatin calcium	50
Salicylic acid	500,000	Sarcosine	1,000
Sertindole	50	Sertraline hydrochloride	600
Simvastatin	30	Sodium benzoate	400,000
Sodium fluoride	150	Spironolactone	600
Sulfamethoxazole	400,000	Sulpiride	50,000
Temazepam	5,000	Theophylline	40,000
Thiamine HCl	50	Topiramate	10,000
Trazodone HCl	6,000	Triamcinolone acetonide	10
Triamterene	9,000	Triazolam	40
Valproic acid	500,000	Vancomycin HCl	100,000
Varenicline	50	Venlafaxine HCl	400
Vitamin B12	50	Vitamin D2	40
Vitamin K1	50	Warfarin	10,000
Ziprasidone	200	Zolpidem hemitartrate	5,000
Zonisamide	40,000	Zopiclone	100
Zuclopenthixol	250		

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%.

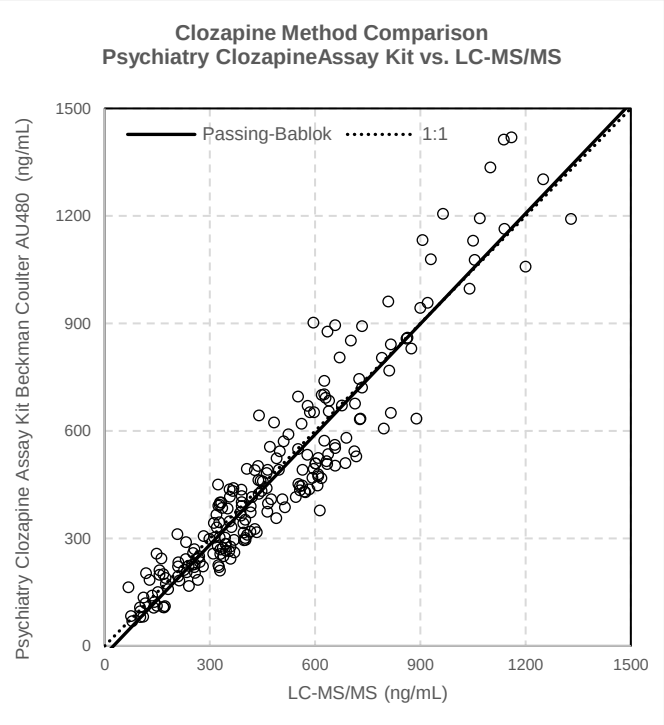
Linearity

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	UK CA	UK Mark
CH REP	Authorized Representative in Switzerland	EC REP	Authorized Representative in the European Community
Made in USA	Made in USA		

For technical assistance:

Contact the Customer Technical Support Center at 1-800-854-3633 (USA & Canada).

In other countries, please contact your local Beckman Coulter representative.

ADDITIONAL INFORMATION

For more detailed information on AU Systems, refer to the appropriate system manual. Since Beckman Coulter does not manufacture the reagent or perform quality control or other tests on individual lots, Beckman Coulter cannot be responsible for the quality of the data obtained which is caused by performance of the reagent, any variation between lots of reagent, or protocol changes by the manufacturer.

SHIPPING DAMAGE

Please notify your Beckman Coulter Clinical Support Center if this product is received damaged.

Beckman Coulter, the stylized logo, and the Beckman Coulter product and service marks mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries.

Saladax BIOMEDICAL

CE EC REP EMERGO EUROPE Westervoortsedijk 60, 6827 AT Arnhem The Netherlands

CH REP Casus Switzerland GmbH Hinterbergstrasse 49 6312 Steinhausen Switzerland

Saladax Biomedical, Inc.
116 Research Dr.
Bethlehem, PA 18015 USA
www.saladax.com/bci_applications/

UK Responsible Person:
Emergo Consulting (UK) Limited
c/o Cr360 – UL International
Compass House, Vision Park Histon
Cambridge CB24 9BZ
United Kingdom

Australian Sponsor
ACRA Regulatory Services Pty Ltd
7/ 84 Poinciana Avenue,
Tewantin, QLD 4565 Australia

New Zealand Sponsor
ACRA Regulatory Services Limited
182 Teasdale Street,
Te Awamutu, 3800, New Zealand

© 2023, Saladax Biomedical, Inc.

Distributed by:
Beckman Coulter, Inc.
250 S. Kraemer Blvd.
Brea, CA 92821 USA