EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
MyCare Psychiatry Clozapine Assay Kit
DECISION SUMMARY

A. DEN Number:

DEN190028

B. Purpose for Submission:

De Novo request for evaluation of class III designation of the MyCare Psychiatry Clozapine Assay Kit

C. Measurand:

Clozapine (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo [b,e] [1,4] diazepine)

D. Type of Test:

Homogeneous nanoparticle agglutination immunoassay

E. Applicant:

Saladax Biomedical, Inc.

F. Proprietary and Established Names:

MyCare Psychiatry Clozapine Assay Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.3245

2. Classification:

   Class II (Special Controls)

3. Product code:

   QKT

4. Panel:

   91- Toxicology

H. Intended Use:

1. Intended use(s):

   See indications for use

2. Indication(s) for use:

   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.

3. Special conditions for use statement(s):

   For prescription use only
   For In Vitro Diagnostic Use Only

4. Special instrument requirements:

   The assay was validated on the Beckman Coulter AU480 Clinical Analyzer

I. Device Description:

The MyCare Psychiatry Clozapine Assay Kit 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. This aggregation is measured at a wavelength of around (b)(4) by automated clinical chemistry analyzers.

The assay contains:

- Reagent 1 (R1) – reaction buffer that contains drug-conjugate in a buffered protein solution
- Reagent 2 (R2) – nanoparticle reagent that contains clozapine-specific monoclonal antibody bound to nanoparticles in a buffered solution

J. Standard/Guidance Document Referenced (if applicable):

- CLSI EP05-A3 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Third Edition, October 2014.

- CLSI EP06-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, April 2003.

- CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - second edition, June 2012.

- CLSI EP25-A Evaluation of Stability of In vitro Diagnostic Reagents; Approved Guideline, September 2009.

K. Test Principle:

Drug-conjugates serve as binding partners to antibodies selective for clozapine which are covalently attached to the surface of nanoparticles. In the absence of free clozapine, this reaction creates large aggregates, resulting in a solution that scatters incident light and leads to an increase in the observed absorption of the solution. When incubated with a sample containing clozapine, the free clozapine in the sample binds to the reagent antibodies, and those antibody-nanoparticle conjugates are no longer available to aggregate, resulting in less scattering of incident light and lower observed absorption of the solution. Thus, maximum absorption occurs with low levels of drug, and minimum absorption occurs with high levels of drug.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A precision study was conducted according to recommendations in CLSI EP05-A3 using 4 human serum sample pools, which were prepared from clinical samples from patients taking clozapine therapy, serum pools spiked with clozapine, and 3 levels of controls. Each sample was assessed in replicates of (b)(4) a day, for (b)(4) days. Samples were assessed using three lots of reagents, calibrators, and controls on two Beckman Coulter AU480 Clinical Analyzers. The results are summarized below.

Sample Results Across All Reagent Lots and Analyzers

Sample	N	Mean (ng/mL)	Within - Run CV	Total CV
Serum pool 1	480	148	4.6%	5.6%
Serum pool 2	480	338	3.3%	4.0%

Sample	N	Mean (ng/mL)	Within - Run CV	Total CV
Serum pool 3	480	577	3.3%	4.0%
Serum pool 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%
Control 1	480	156	4.5%	5.4%
Control 2	480	474	3.7%	4.4%
Control 3	480	945	3.8%	4.5%

b. Linearity/assay reportable range:

A study was conducted to evaluate linearity across the measuring range of the assay following the CLSI EP06-A guideline. Samples with 11 different clozapine levels were evaluated: 55, 132, 169, 352, 491, 588, 821, 1003, 1214, 1467, and 1554 ng/mL. Two samples were prepared by pooling human serum specimens that were then spiked with clozapine to low and high clozapine concentrations, which were then mixed to produce samples with the concentrations tested. Each sample was tested in five replicates using three lots of reagents, calibrators, and controls and two Beckman Coulter AU480 clinical analyzers. The observed values were plotted against the expected values and linear regression analysis was performed. The linear regression results from the worst performing lot of three lots are shown below.

Claimed Range	Slope	Intercept	R
68-1500 ng/mL	0.920 (0.910 to 0.930)	4.1 (1.4 to 6.8)	0.9985

The 95% confidence intervals are shown for slope and intercept.

These results support the claimed measuring range of 68 to 1500 ng/mL for clozapine.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability

The MyCare Psychiatry Calibrator Kit is traceable to a certified USP clozapine reference standard. Master calibrators are prepared by (b)(4) of this

certified reference material in buffer and verified by LC-MS/MS.

d. *Detection limit:*

Detection limits were assessed according to recommendations in CLSI EP17-A2.

The limit of blank (LOB) was determined using the non-parametric "classical approach" using (b)(4) individual normal (b) (4) serum samples containing no clozapine. Each of the (b)(4) serum samples were tested in duplicate on three different days on two different analyzers for n=(b) (4), using (b)(lots of reagents, calibrators and controls. The 95th percentile of each n = (b) (4) data set was calculated, and the highest 95th percentile value from among the three n = (b) (4) data sets was determined. The sponsor determined the LOB to be (b) (4) ng/mL.

The limit of detection (LOD) was determined using the variant (non-parametric analysis) "classical approach" with a beta of (b) (4) individual normal human serum samples spiked with clozapine at 15, 20, 25, 30, and 35 ng/mL. Each of the (b)(serum samples were tested in duplicate on three different days on (b)(4) different analyzers for n=(b) (4), using (b)(lots of reagents, calibrators and controls. The LOD was determined using the highest median value observed at the lowest spiking level for which each of the three n = (b) (4) data sets associated with that spiking level had (b) (4) of results below the LOB. The sponsor determined the LOD to be 39 ng/mL.

The limit of quantitation (LOQ) was determined by 4 individual normal human serum samples spiked with clozapine at (b)(targeted concentrations: 40, 50, 60, 70 and 80 ng/mL. Each of the (b)(serum sample was tested n = (b)(on (b) (4) different days on (b)(4) analyzer, using (b)(lots of reagents/calibrators/controls, for n=(b) (4) (results combined across all lots). The LOQ was defined as the specimen concentration where the total allowable error was ≤ 35% when calculated according to the Westgard model. The sponsor determined the LOQ to be 68 ng/mL.

e. *Analytical specificity:*

Potentially Cross-Reacting Substances

Cross-reactivity was evaluated by adding each potentially cross-reacting clozapine metabolite into a human serum pool prepared from multiple individual human serum samples. The sponsor tested the three metabolites in the presence of clozapine at 350 ng/mL. Testing was conducted using one lot of reagents, one lot of calibrators, and two analyzers. Samples were tested in (b) (4) replicates. The formula used for calculating percent cross reactivity was:

(b) (4)

The results are summarized below. The sponsor did not observe cross-reactivity

exceeding ±10%:

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

Endogenous and Exogenous Interfering Substances:

Interference studies were conducted based on the CLSI guideline EP07-A2. Potentially interfering substances (test sample) or solvent (control sample) were spiked into human serum sample pools with clozapine at 350 and 600 ng/mL. Each sample was tested at a minimum of 5 runs using one lot of reagent on two analyzers. The percentage difference between the measurement of clozapine in the test sample and the control sample was calculated. The sponsor considered a percent difference between test samples and control samples of > ±10% to be clinically significant interference. None of the substances in the following tables (endogenous or exogenous substances) were found to lead to clinically significant interference (> ±10%) for the device.

Endogenous Substances

Interfering Substance	Concentration Tested
Bilirubin	18.18 mg/dL
Hemolysate	1,050 mg/dL
Human IgG (HIgG)	12.5 g/dL
Human Serum Albumin (HSA)	10.9 g/dL
Rheumatoid Factor (RF)	508 IU/mL
Triglycerides	2,585.87 mg/dL

Exogenous Interferences

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	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
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
Escitalopram	200	Estradiol	10

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
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	Hydrochloro-thiazide	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
Pantothenic acid	1,800	Paroxetine	1,200
Penicillin V	42,000	Perazine	1,400

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

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison study:

A method comparison study was conducted using the MyCare Psychiatry Clozapine Assay Kit on the Beckman Coulter AU480 by testing clinical samples that were collected at six different U.S. clinical sites from patients receiving clozapine therapy. Results from the MyCare Psychiatry Clozapine Assay Kit were compared to results from a validated clozapine LC-MS/MS method. Deming regression analysis was performed with (b)(4) samples, resulting in the following line equation (with 99% confidence intervals shown) from a representative lot:

y = 1.037 (0.939 to 1.135) x - 27.8 (-78.3 to +22.7); R = 0.9269

A summary of the device accuracy (compared to the LC-MS/MS comparator method) at different concentration ranges throughout the claimed measuring interval is shown below.

	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

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

The sponsor provided a discussion of the clinical use of therapeutic drug monitoring for clozapine in patients with treatment-resistant schizophrenia, as provided in the U.S. clinical practice guideline, "The American Psychiatric Association Practice Guideline for the Treatment of Patients with Schizophrenia" (2020).

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

There is no definitive therapeutic range for clozapine, as this range may be different for each patient.

N. Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

O. Identified Risks to Health and Mitigation Measures

Identified Risks to Health	Mitigation Measures
Incorrect test results	Certain design verification and validation activities Certain labeling information
Incorrect interpretation of test results	Certain design verification and validation activities Certain labeling information

P. Benefit/Risk Determination

Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

Summary of the Assessment of Benefit
There is currently no available FDA cleared or approved device for determining Clozapine

blood concentrations. Toxic side effects from Clozapine include seizures, myocarditis, agranulocytosis, and other severe adverse effects, and Clozapine currently requires a REMS (Risk Evaluation and Mitigation Strategy) to ensure the benefits of the drug outweigh the risk of severe neutropenia. The availability of the test may aid in the management of patients with treatment-resistant schizophrenia under various clinical scenarios as described in the American clinical practice guideline, "The American Psychiatric Association Practice Guideline for the Treatment of Patients with Schizophrenia" (2020) (e.g. "while the dose of clozapine is being titrated, it is useful to obtain blood levels of clozapine").

Summary of the Assessment of Risk

In the setting of poor or incomplete drug response, a falsely low clozapine level may increase the risk of a prescriber falsely concluding that the outcome is due to sub-therapeutic levels which may prompt an unnecessary increase in drug dose and increase the risk of adverse events such as seizures. In the setting of potential toxicity, a falsely low level may delay clozapine dose de-escalation, increasing the risk of persistent adverse events and severe adverse events. In the setting of poor or incomplete drug response, a falsely high level may increase the risk of a prescriber incorrectly assuming that the patient was receiving an adequate dose and continue a suboptimal dose or conclude that the patient was not responding to clozapine and prescribe alternate therapies that may be less effective for the patient, increasing the risk of continued psychological distress from persistent symptoms, poorer social and occupational functioning, hospitalization, or transfer to residential settings. Additionally, if a patient's clozapine level is falsely elevated, a prescriber may not fully investigate the possibility of suboptimal dosing, which could result in a missed opportunity to identify and rectify conditions that could lead to suboptimal dosing or to consider treatment alternatives that are more acceptable to the patient. Falsely elevated levels may lead to a prescriber deciding to decrease the dose or discontinue medication, even if the patient were tolerating the medication and had experienced benefit, which could increase the risk of relapse, functional impairment, or transition to higher levels of care.

Summary of the Assessment of Benefit-Risk

General controls are insufficient to mitigate the risks of the device. However, the probable clinical benefits outweigh the probable risks for the assay, considering the mitigation of the risks provided for in the special controls. Device design verification and validation, including precision, method comparison, interference, and cross-reactivity studies will help ensure that the device functions as intended and mitigate the risk of falsely low or falsely high test results. A limitation statement conveying that results from the assay alone should not be used in making treatment decisions will be included in the labeling, as a mitigation against the risk of incorrect interpretation of results. Overall, the probable benefits outweigh the probable risks of incorrect test results or incorrect interpretation of test results for the proposed indications for use, in light of the special controls and general controls.

Q. Conclusion

The De Novo request for the MyCare device is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code: QKT
Device Type: Clozapine test system
Class: II (special controls)
Regulation: 21 CFR 862.3245