REF RSP-RGT

MyCare Psychiatry Total Risperidone Assay Kit

INDICATIONS FOR USE

The MyCare Psychiatry Total Risperidone Assay Kit is intended for the *in vitro* quantitative measurement of risperidone and paliperidone (9-hydroxyrisperidone) in human serum using automated clinical chemistry analysers. Measurements obtained are used for monitoring patient adherence to risperidone or paliperidone therapy to help ensure appropriate treatment.

SUMMARY AND EXPLANATION OF THE TEST

Risperidone (3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl] ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one) is a benzisoxazole derivative, atypical antipsychotic agent used in the treatment of schizophrenia, acute manic or mixed episodes associated with bipolar disorder 1, and irritability associated with autistic disorder.[1,2]

Paliperidone (3-[2-[4-(6-fluoro-1,2-benzoxazol-3-yl)piperidin-1-yl]ethyl]-9-hydroxy-2-methyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrimidin-4-one) is a benzisoxazole derivative, atypical antipsychotic agent used in the treatment of schizophrenia and schizoaffective disorder.[3,4]

The major metabolite of risperidone, paliperidone, is also pharmaceutically active. The therapeutic effect of risperidone is due to the total exposure to both risperidone and the active metabolite i.e. total risperidone.[5] The total risperidone assay measures the total active risperidone in patient serum: risperidone plus paliperidone. Nonadherence to medication is well known for patients with severe mental illness.[6] While adherence to medication is critical to successful treatment outcomes, adherence is also least likely to be accurately assessed by clinicians.[7,8] Measurement of risperidone and paliperidone provides clinicians with objective evidence of concentrations that may be related to patient adherence.[9]

The total risperidone assay (US Patent 8,088,594) is a homogenous two reagent nanoparticle agglutination assay used for detection of risperidone and paliperidone 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 analysers.

REAGENTS

MyCare Psychiatry Total Risperidone Assay Kit **REF** RSP-RGT	Quantity x Volume
Reagent 1 **R1** Reaction buffer that contains drug-conjugate, protein, and buffer	1 x 10.0mL
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 total risperidone 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 total risperidone 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. Improper storage of reagents can affect assay performance.

SPECIMEN COLLECTION AND HANDLING

Serum is required. Trough or C_{min} samples at steady state have been recommended for testing antipsychotics.[9,10] After one week of treatment on the same dose, collect samples 20 – 24 hours (daily dosing) or 9 - 12 hours (twice daily dosing) after the last dose.[11,12] For long lasting injectables collect the sample before the next dose.[13]

Prepare serum within 3 days of blood collection. Blood and serum samples may be stored at room temperature or 2 - 8°C. Store serum 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 RSP-RGT – MyCare Psychiatry Total Risperidone 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 total risperidone assay. 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 for risperidone from ng/mL is 2.44 x ng/mL = 1 nmol/L. The conversion factor for paliperidone from ng/mL is 2.35 x ng/mL = 1 nmol/L.

LIMITATIONS OF THE PROCEDURE

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

Bromperidol when tested at 100 ng/mL had an assay bias of ≥ 38%. Droperidol when tested at 200 ng/mL had an assay bias of ≥ 63%. Haloperidol when tested at 50 ng/mL had an assay bias of ≥ 17%. Sertindole when tested at 300 ng/mL had an assay bias of ≥ 17%. Elevated levels of risperidone may be seen in patients administered bromperidol, droperidol, haloperidol, or sertindole. Elevated levels of paliperidone may be seen in patients administered bromperidol, droperidol, haloperidol, or sertindole.

Paliperidone is the active metabolite of risperidone. For patients co-administrated paliperidone and risperidone, paliperidone will be quantitated as total risperidone.

EXPECTED VALUES

The therapeutic range for total risperidone or paliperidone in serum is not fully established. A therapeutic range from 20 to 60 ng/mL has been proposed for both risperidone and paliperidone.[9] Measured concentrations for adherent patients at steady state are expected to be in the measuring range of the assay.[14] Therapeutic drug monitoring of total risperidone or paliperidone has been recommended because of high interpatient variability, unpredictable response, and the importance of adherence for successful therapy.[9] The complexity of the clinical state, individual differences in sensitivity, and co-administered medications may contribute to different requirements for optimal total risperidone and paliperidone 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 total risperidone 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 EP5-A3.[15] Three control kit controls, three risperidone spiked pools (Serum 1, 2, 3) and two pools of clinical samples (Clinical 1, 2) were tested.

Sample	N	Mean (ng/mL)	Repeatability	Within-Laboratory
			CV	CV
Control 1	80	36	2.8%	3.7%
Control 2	80	65	2.1%	2.8%
Control 3	80	99	2.5%	3.3%
Serum 1	80	21	3.3%	5.0%
Serum 2	80	59	2.4%	4.2%
Serum 3	80	78	3.3%	6.0%
Clinical 1	80	22	3.0%	4.2%
Clinical 2	80	58	3.1%	3.8%

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

The lower limits of quantitation and detection were established using CLSI guideline EP17-A2.[16]

LoQ

The LoQ was determined with an accuracy goal at the LoQ of ≤ 35% total error (Westgard model). The LoQ of the total risperidone assay is 16 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 total risperidone assay is 7 ng/mL.

Result Reporting

Each laboratory should determine reporting criteria for total risperidone concentrations. The following suggestion from CLSI EP17-A2 may be appropriate:[16]

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 total risperidone assay is 16 – 120 ng/mL.

Specificity

Metabolism

Risperidone is extensively metabolized in the liver by CYP2D6 and to a lesser extent by CYP3A4.[1] The biotransformation by CYP2D6 gives the major metabolite (±) 9-hydroxyrisperidone (paliperidone), both enantiomers of which are as active as the parent drug. The therapeutic effect of risperidone is due to the total exposure to both risperidone and the active metabolite.

There are two minor metabolites of risperidone in serum. 7-hydroxyrisperidone occurs as 1-5% of parent drug.[17] The minor N-desalkyl-risperidone metabolite has been reported to occur at 10 – 13% of the parent drug.[17]

Paliperidone itself is not extensively metabolized.[18] No metabolites have been detected in plasma and paliperidone accounts for 97% of the area under the curve at 24 hours.[19]

Specificity for the following metabolites was tested in the absence and presence of risperidone and paliperidone at 20, 60, and 120 ng/mL.

Compound	Tested at (ng/mL)	Cross-Reactivity
7-hydroxyrisperidone	10	< 60%
N-desalkyl risperidone	20	< 5%

Interfering Substances

Testing of interferents was conducted according to CLSI guidelines for interferences.[20-22] No significant assay bias was observed from samples with the following endogenous interferents at the given levels.

Interferent	Level	
Rheumatoid Factor	420 IU/mL	
Human Serum Albumin	10.8 g/dL	108 g/L
Human Immunoglobulin G	12.0 g/dL	120 g/L
Icteric Interference	27.85 mg/dL	476 µmol/L
Lipemic Interference	1,297 mg/dL	15 mmol/L
Hemolysate	1,050 mg/dL	

Cross-reactivity

Specificity for the following cross-reactants was tested in the absence and presence of risperidone and paliperidone at 20, 60, and 120 ng/mL.

Cross-reactivity was tested according to CLSI guidelines for interferences.[20-22] The following compounds did not interfere with the total risperidone assay: cross reactivity was ≤ 5% or the assay bias was ≤ 15%.

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	Amoxapine	2,900
Amoxicillin	80,000	S (+)-amphetamine	1,000
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
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
Clotrimazole	50	Clozapine	1,800
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	Donepezil	50,000
Doxycycline HCl	35,000	Duloxetine	200
Erythromycin	138,000	Escitalopram	200
Estradiol	10	Eszopiclone	300
Ethanol	10,000,000	Famotidine	2,500

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

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
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	Rosuvastatin calcium	200
Salicylic acid	500,000	Sarcosine	1,500
D-Serine	100,000	Sertraline hydrochloride	1,000
Simvastatin	1,700	Sodium benzoate	400,000
Sodium fluoride	900	Spironolactone	600

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
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	12,000
Zopiclone	200	Zuclopenthixol	300

Recovery

Patients on risperidone therapy have both risperidone (RSP) and the active metabolite paliperidone (PAL) in their serum. Therefore, to assess recovery of the total risperidone assay, risperidone and the active metabolite paliperidone were spiked together into four individual normal risperidone-free sera. The percent recovery was determined by dividing the observed concentration of each sample by the expected concentration of added risperidone plus paliperidone.

Mean Percent Recovery

Theoretical ng/mL	RSP:PAL ratio	Percent Recovery	RSP:PAL ratio	Percent Recovery
20	4:1	90 – 120	1:4	90 – 120
60	4:1	90 – 108	1:4	92 – 115
120	4:1	90 – 110	1:4	95 – 115

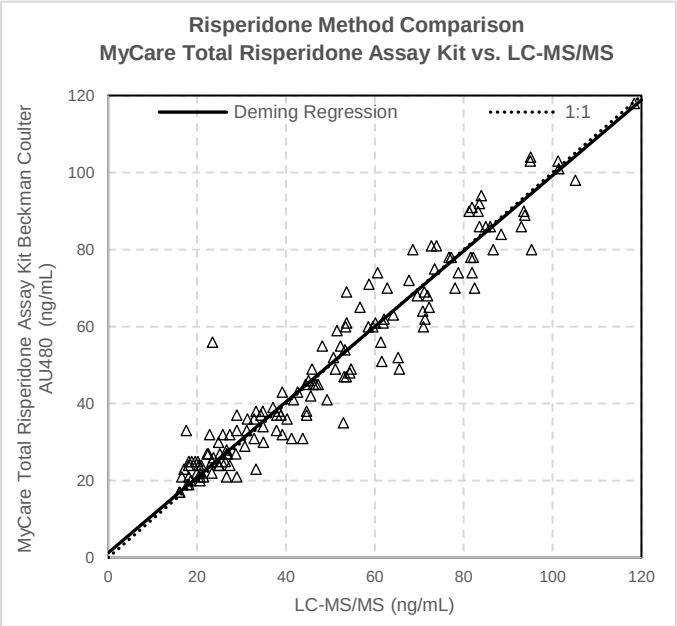
Linearity

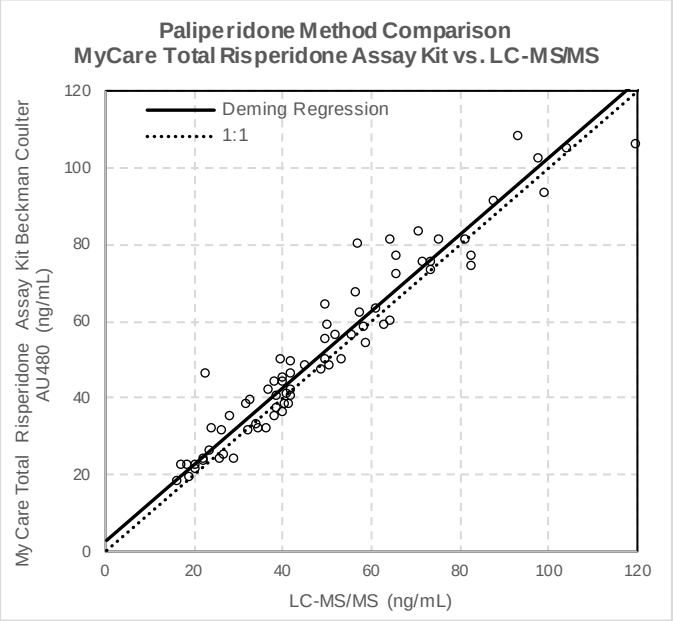
The linearity of the total risperidone assay was verified according to CLSI guideline EP6-A.[23] Eleven linearity samples covering the measuring range were prepared in human serum spiked with risperidone and eleven linearity samples covering the measuring range were prepared in human serum spiked with paliperidone. Deviation from linearity (n=5) for eleven sample with risperidone or paliperidone was < 6%. The assay was linear across the measuring range from 16 – 120 ng/mL.

Method Comparison

Results of the total risperidone assay were compared to a validated LC-MS/MS, using samples from patients taking risperidone or paliperidone according to CLSI guideline EP09-A3.[24] Deming regression analysis was performed with 146 risperidone patient samples and 119 paliperidone patient samples. Results are shown for one lot.

Deming Regression Statistics Total Risperidone Assay vs. LC-MS/MS		
Statistic	Risperidone Samples	Paliperidone Samples
Slope	0.98	1.00
Intercept	1	3
Correlation Coefficient (R)	0.96	0.94
N	146	119
Concentration Range (LC-MS/MS)	16 – 118 ng/mL	16 – 120 ng/mL





References

1. Janssen Pharmaceuticals, Inc. Risperdal® (risperidone) Prescribing information. 2017.
2. Janssen Pharmaceuticals, Inc. Risperidal Consta® (risperidone) Long-acting Injection Prescribing Information. 2017.
3. Janssen Pharmaceuticals, Inc. Invega® (paliperidone) Prescribing information. 2017.
4. Janssen Pharmaceuticals, Inc. Invega Sustenna® (paliperidone palmitate) extended-release injectable suspension Prescribing Information. 2017.
5. Seto K, Dumontet J, Ensom MH. Risperidone in schizophrenia: is there a role for therapeutic drug monitoring? *Ther Drug Monit*. 2011;33(3):275-283.
6. 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.
7. 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.
8. Haddad PM, Brain C, Scott J. Nonadherence with antipsychotic medication in schizophrenia: challenges and management strategies. *Patient Relat Outcome Meas*. 2014;5:43-62.
9. Hiemke C, Bergemann N, Clement HW, et al. Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017. Pharmacopsychiatry. 2018:51:9-62.
10. Bowskill SVJ, Handley SA, Fisher DS, Flanagan RJ, Patel MX. Risperidone and Total 9-Hydroxyrisperidone in Relation to Prescribed Dose and Other Factors: Data From a Therapeutic Drug Monitoring Service, 2002–2010. *Ther Drug Monit*. 2012;34(3):349-355.
11. Lostia AM, Mazzarini L, Pacchiarotti I, et al. Serum levels of risperidone and its metabolite, 9-hydroxyrisperidone: correlation between drug concentration and clinical response. *Ther Drug Monit.* 2009;31(4):475-481.
12. Aravagiri M, Marder SR, Nuechterlein KH, Gitlin MJ. Intra- and interindividual variations in steady-state plasma concentrations of risperidone and 9-hydroxyrisperidone in schizophrenic patients treated chronically with various doses of risperidone. *Ther Drug Monit*. 2003;25(6):657-664.
13. Gefvert O, Eriksson B, Persson P, et al. Pharmacokinetics and D2 receptor occupancy of long-acting injectable risperidone (Risperdal Consta) in patients with schizophrenia. *Int J Neuropsychopharmacol*. 2005;8(1):27-36.
14. Melkote R, Singh A, Vermeulen A, Remmerie BM, Savitz A. Relationship between Antipsychotic Blood Levels and Treatment Failure during the CATIE Study. International Congress on Schizophrenia Research. 2017;Poster.
15. 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.
16. 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.
17. Mannens G, Huang ML, Meuldermans W, Hendrickx J, Woestenborghs R, Heykants J. Absorption, metabolism, and excretion of risperidone in humans. Drug Metab Dispos. 1993;21(6):1134-1141.
18. Spina E, Cavallaro R. The pharmacology and safety of paliperidone extendedrelease in the treatment of schizophrenia. Expert Opin Drug Saf. 2007;6(6):651-662.
19. Vermeir M, Naessens I, Remmerie B, et al. Absorption, metabolism, and excretion of paliperidone, a new monoaminergic antagonist, in humans. Drug Metab Dispos. 2008;36(4):769-779.
20. CLSI. Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition CLSI document EP7-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
21. CLSI. Interference Testing in Clinical Chemistry. 3rd ed. CLSI guideline EP07. Wayne PA: Clinical and Laboratory Standards Institute; 2018.
22. CLSI. Supplemental Tables for Interference Testing in Clinical Chemistry. 1st ed. CLSI supplement EP37. Wayne PA: Clinical and Laboratory Standards Institute; 2018.
23. NCCLS. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. NCCLS document EP6-A. Wayne, PA: NCCLS; 2003.
24. 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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