December 21, 2022

Microgenics Corporation
Pranjali Shinde
Senior Regulatory Affairs Specialist
46500 Kato Road
Fremont, CA 94538

Re: K213875
Trade/Device Name: DRI ™ Tricyclics Serum Tox Assay
Regulation Number: 21 CFR 862.3910
Regulation Name: Tricyclic antidepressant drugs test system
Regulatory Class: Class II
Product Code: LFH
Dated: August 19, 2022
Received: August 23, 2022

Dear Pranjali Shinde:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR

803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Paula Caposino -S

Digitally signed by Paula Caposino -S
Date: 2022.12.21 11:51:25 -05'00'

Paula Caposino, Ph.D.
Acting Deputy Division Director
Division of Chemistry
and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K213875

Device Name
DRI™ Tricyclics Serum Tox Assay

Indications for Use *(Describe)*

The DRI™ Tricyclics Serum Tox Assay is a homogeneous enzyme immunoassay intended for the qualitative and/or semiquantitative determination of the presence of tricyclic antidepressants (TCAs) in human serum, plasma, or urine of patients at a cutoff concentration of 300 ng/mL in patients suspected of drug overdose.

The semi-quantitative mode is for the purpose of enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) or permitting laboratories to establish quality control procedures.

The assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) is the preferred confirmatory method.

Clinical and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

For In Vitro Diagnostic Use Only.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

510(k) Summary

510(k) Number: K213875

This 510(k) Summary of Safety and Effectiveness is being submitted in accordance with the requirements of Safe Medical Device Act of 1990 and 21 CFR 807.92.

A. Device Information

Sponsor:	Microgenics Corporation Thermo Fisher Scientific 46500 Kato Road Fremont, CA 94538 Phone: 510-979-5000 FAX: 510-979-5002
Correspondent Contact Information:	Pranjali Shinde Microgenics Corporation Thermo Fisher Scientific 46500 Kato Road Fremont, CA-94538 Email: *Pranjali.Shinde@Thermofisher.com* Phone: 213-344-9952 Fax: 510-979-5002
Device Common Name:	Tricyclics Serum Tox Assay
Trade or Proprietary Name	DRI™ Tricyclics Serum Tox Assay
Brand Name	DRI™ Tricyclics Serum Tox Assay
Candidate Device Product Code, Classification, Classification Name & Panel	LFH, Class II, 21 CFR 862. 3910 – Tricyclic antidepressant drugs test system, 91 – Toxicology

Predicate Device Information

Predicate Device:	Tricyclic Serum Tox EIA Assay
Predicate Device Manufacturer:	Microgenics Corporation
Predicate Device Common Name	Tricyclic Serum Tox Assay
Predicate Device Premarket Notification #:	K983268
Predicate Device Product Code, Classification, Classification Name & Panel	LFH, Class II, 21 CFR 862. 3910 – Tricyclic antidepressant drugs test system, 91 – Toxicology

B. Date Summary Prepared

November 14, 2022

C. Description of Device

The DRI™ Tricyclics Serum Tox Assay is a homogeneous enzyme immunoassay using ready to-use liquid reagents. Specific tricyclic antibodies were used to detect most tricyclic antidepressants in serum, plasma, or urine. The test is based on the competition of an enzyme, glucose-6-phosphate dehydrogenase (G6PDH), labeled-drug and the drug from the sample for a fixed amount of specific antibody binding sites. In the absence of the drug from the sample, the specific antibody binds the enzyme-labeled drug and the enzyme activity is inhibited. This phenomenon creates a direct relationship between drug concentration in the sample and the enzyme activity. The enzyme activity is determined spectrophotometrically at 340 nm by measuring its ability to convert nicotinamide adenine dinucleotide (NAD) to NADH.

The DRI™ Tricyclics Serum Tox assay is supplied as a two liquid reagent kit (Reagent A and Reagent E). They are bottled separately within the same kit, see details below:

- **Antibody/Substrate Reagent (Reagent A):** Contains polyclonal anti-tricyclics antibodies (sheep), glucose-6- phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as a preservative.
- **Enzyme Conjugate Reagent (Reagent E):** Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with nortriptyline in Tris buffer with sodium azide as a preservative.

CI. Intended Use

The DRI™ Tricyclics Serum Tox Assay is a homogeneous enzyme immunoassay intended for the qualitative and/or semiquantitative determination of the presence of tricyclic antidepressants (TCAs) in human serum, plasma, or urine of patients at a cutoff concentration of 300 ng/mL in patients suspected of drug overdose.

The semi-quantitative mode is for the purpose of enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) or permitting laboratories to establish quality control procedures.

The assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) is the preferred confirmatory method.

Clinical and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

For *In Vitro Diagnostic* Use Only.

E. Comparison to Predicate Device

Characteristics	Candidate Device: DRI™ Tricyclics Serum Tox Assay (K213875)	Predicate Device: Tricyclic Serum Tox EIA Assay (K983268)
Intended Use	The DRI™ Tricyclics Serum Tox Assay is a homogeneous enzyme immunoassay intended for the qualitative and/or semiquantitative determination of the presence of tricyclic antidepressants (TCAs) in human serum, plasma, or urine of patients at a cutoff concentration of 300 ng/mL in patients suspected of drug overdose. The semi-quantitative mode is for the purpose of enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) or permitting laboratories to establish quality control procedures. The assay provides only a preliminary analytical test result. A more specific alternative chemical method must be used to obtain a confirmed analytical result. Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) is the preferred confirmatory method. Clinical and professional judgment should be applied to any drug of abuse test result,	Same

	particularly when preliminary positive results are used. For *In Vitro Diagnostic* Use Only.	
Operating Principle (Technology)	DRI	Same
Measured Analyte	Nortriptyline	Same
Test Matrix	Serum, Plasma, Urine	Same
Cut-off Levels	300 ng/mL	Same
Methodology	Homogeneous Enzyme Immunoassay	Same
Reagents Form	Liquid ready-to-use.	Same
Antibody	Sheep polyclonal antibodies	Monoclonal antibodies
Storage	2–8 °C until expiration date.	Same
Principal Operator	Trained professionals	Same
Calibrator Levels for Semi-Quant	5-point Calibrator	5-point Calibrator

F. Test Principle

The DRI™ Tricyclics Serum Tox Assay is a homogeneous enzyme immunoassay using ready-to use liquid reagents. The assay uses specific tricyclic antibodies, which can detect most tricyclic antidepressants in serum, plasma, or urine. The DRI technology is based on the competition of an enzyme glucose-6-phosphate dehydrogenase (G6PDH) labeled-drug and the drug from the serum, plasma, or urine sample for a fixed amount of specific antibody binding sites. In the presence of free drug from the sample, the free drug occupies the antibody binding sites, allowing the drug-labeled G6PDH to interact with the substrate, resulting in enzyme activity. In the absence of drug from the sample, the specific antibody binds to the drug labeled with G6PDH and the enzyme activity is inhibited. This phenomenon creates a direct relationship between the drug concentration in the serum, plasma, or urine and the enzyme activity. The enzyme G6PDH activity is determined spectrophotometrically at 340 nm by measuring its ability to convert nicotinamide adenine dinucleotide (NAD) to NADH.

G. Summary of Supporting Data

Analytical Performance:
The performance was evaluated on Architect c4000 clinical chemistry analyzer.

a) Precision
Precision studies were performed in accordance with CLSI Guideline *EP05-A3*.

Samples were prepared by spiking Nortriptyline in both drug-free serum and drug-free urine at the cutoff, 25%, 50%, 75%, and 100% above and below the cutoff.

Repeatability:
The study was conducted for 20 days with 2 runs per day, 2 replicates per run in both qualitative and semi-quantitative modes with 1 lot of reagent, calibrators, and controls. There were 80 replicates for each level of precision samples. Serum and urine samples were run separately. The results of the precision study (Repeatability) are presented in the table below.

Table 1: Precision study data (Repeatability) in qualitative and semi-quantitative mode for 300 ng/mL cutoff – Serum

Spiked Concentration (ng/mL)	% of Cutoff	Total Precision (n=80)		
		# of Determinants	Qualitative Immunoassay Results (Negative/Positive)	Semi-Quantitative Immunoassay Results (Negative/Positive)
0	-100	80	80/0	80/0
75	-75	80	80/0	80/0
150	-50	80	80/0	80/0
225	-25	80	80/0	80/0
300	100	80	10/70	19/61
375	+25	80	0/80	0/80
450	+50	80	0/80	0/80
525	+75	80	0/80	0/80
600	+100	80	0/80	0/80

Table 2: Precision study data (Repeatability) in qualitative and semi-quantitative mode for 300 ng/mL cutoff – Urine

Spiked Concentration (ng/mL)	% of Cutoff	Total Precision (n=80)		
		# of Determinants	Qualitative Immunoassay Results (Negative/Positive)	Semi-Quantitative Immunoassay Results (Negative/Positive)
0	-100	80	80/0	80/0
75	-75	80	80/0	80/0
150	-50	80	80/0	80/0
225	-25	80	80/0	80/0
300	100	80	68/12	73/7
375	+25	80	0/80	0/80
450	+50	80	0/80	0/80
525	+75	80	0/80	0/80
600	+100	80	0/80	0/80

Reproducibility:

The study was conducted for 5 days with 1 run per day, 5 replicates per run in both qualitative and semi-quantitative modes with 2 lots of reagents on 3 different instruments. Serum and urine samples were run separately. The results of the precision study (Reproducibility) are presented in the table below.

Table 3: Precision study data (Reproducibility) in qualitative and semi-quantitative mode for 300 ng/mL cutoff – Serum

Spiked Concentration (ng/mL)	% of Cutoff	Total Precision (n=75)		
		# of Determinants	Qualitative Immunoassay Results (Negative/Positive)	Semi-quantitative Immunoassay Results (Negative/Positive)
0	-100	75	75/0	75/0
75	-75	75	75/0	75/0
150	-50	75	75/0	75/0
225	-25	75	75/0	75/0
300	100	75	30/45	29/46
375	+25	75	0/75	0/75
450	+50	75	0/75	0/75
525	+75	75	0/75	0/75
600	+100	75	0/75	0/75

Table 4: Precision study data (Reproducibility) in qualitative and semi-quantitative mode for 300 ng/mL cutoff – Urine

Spiked Concentration (ng/mL)	% of Cutoff	Total Precision (n=75)		
		# of Determinants	Qualitative Immunoassay Results (Negative/Positive)	Semi-quantitative Immunoassay Results (Negative/Positive)
0	-100	75	75/0	75/0
75	-75	75	75/0	75/0
150	-50	75	75/0	75/0
225	-25	75	75/0	75/0
300	100	75	61/14	66/9
375	+25	75	0/75	0/75
450	+50	75	0/75	0/75
525	+75	75	0/75	0/75
600	+100	75	0/75	0/75

b) Spike Recovery

The spike recovery study was performed using 20 replicates. Samples were prepared by spiking Nortriptyline in both drug-free serum and drug-free urine at 225 ng/mL, 300 ng/mL, 375 ng/mL. The study demonstrated the accuracy of spike recovery at low control (225 ng/mL) and high control (375 ng/mL) in both qualitative and semi-quantitative mode. Serum and urine samples were run separately. The results of the study are presented in the table below.

Table 5: Spike Recovery in qualitative and semi-quantitative mode for 300 ng/mL cutoff – Serum

Replicates	Low Control: 225 ng/mL (-25% of cutoff) (n=20)	High Control: 375 ng/mL (+25% of cutoff)(n=20)
1	Negative	Positive
2	Negative	Positive
3	Negative	Positive
4	Negative	Positive
5	Negative	Positive
6	Negative	Positive
7	Negative	Positive
8	Negative	Positive
9	Negative	Positive
10	Negative	Positive
11	Negative	Positive
12	Negative	Positive
13	Negative	Positive
14	Negative	Positive
15	Negative	Positive
16	Negative	Positive
17	Negative	Positive
18	Negative	Positive
19	Negative	Positive
20	Negative	Positive
Overlap	No	No
Relative to C/O	All 20 below C/O	All 20 above C/O

Table 6: Recovery in qualitative and semi-quantitative mode for 300 ng/mL cutoff – Urine

Replicates	Low Control: 225 ng/mL -25% of cutoff (n=20)	High Control: 375 ng/mL +25% of cutoff (n=20)
1	Negative	Positive
2	Negative	Positive
3	Negative	Positive
4	Negative	Positive

Replicates	Low Control: 225 ng/mL -25% of cutoff (n=20)	High Control: 375 ng/mL +25% of cutoff (n=20)
5	Negative	Positive
6	Negative	Positive
7	Negative	Positive
8	Negative	Positive
9	Negative	Positive
10	Negative	Positive
11	Negative	Positive
12	Negative	Positive
13	Negative	Positive
14	Negative	Positive
15	Negative	Positive
16	Negative	Positive
17	Negative	Positive
18	Negative	Positive
19	Negative	Positive
20	Negative	Positive
Overlap	No	No
Relative to C/O	All 20 below C/O	All 20 above C/O

c) Dilution Linearity

The Dilution Linearity study is performed using the CLSI guideline *CLSI EP06-A.*

To demonstrate the dilution linearity for purposes of sample dilution and quality control of the entire assay range, drug-free serum or drug-free urine were spiked using Nortriptyline and diluted with drug-free serum or drug-free urine, respectively to generate 9 levels between 0 and 1000 ng/mL. Each sample was run in replicates of 5 in semi-quantitative mode and the average was used to determine percent recovery compared to the expected target value. Serum and urine samples were run separately. The study results are presented in the table below.

Table 7: Dilution linearity data – Serum

Level #	Expected Nortriptyline Values (ng/mL)	Serum	
		Observed Values (ng/mL)	Recovery (%)
1	0	7	N/A
2	125	121	97
3	250	242	97
4	375	385	103
5	500	479	96
6	625	720	115
7	750	839	112
8	875	913	104
9	1000	1046	105

Table 8: Dilution linearity data – Urine

Level #	Expected Nortriptyline Values (ng/mL)	Urine	
		Observed Values (ng/mL)	Recovery (%)
1	0	8	N/A
2	125	148	118
3	250	271	108
4	375	393	105
5	500	470	94
6	625	620	99
7	750	741	99
8	875	838	96
9	1000	946	95

d) Method Comparison and Accuracy

The method comparison study was performed in accordance with CLSI guideline *CLSI EP09c-A3.*

One (1) replicate of each of the 61 negative patient samples for serum and 50 negative samples for urine and 56 positive patient samples for serum and 50 positive samples for urine were analyzed in both qualitative and semi-quantitative modes. Serum and urine samples were run separately. The results were compared to LC-MS/MS lab testing.

Serum and urine samples were run separately. The results of the study are presented in the tables below.

Table 9: Stratified data comparing immunoassay in qualitative mode with LC-MS/MS – Serum

DRI TCA ST Assay Results	Negative by LC-MS/MS	< 50% of Cutoff concentration by LC-MS/MS (< 150 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (150-299 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (300-450 ng/mL)	High Positives (Greater than 50% above cutoff concentration (> 450 ng/mL)
Positive	0	1 [1]	0	31	25
Negative	1	35	24	0	0
% Negative Sample Agreement			98% or (60/61)		
% Positive Sample Agreement			100% or (56/56)		
% Total Sample Agreement			99% or (116/117)		

[1] Refer to Table 11 for discordant samples of serum.

Table 10: Stratified data comparing immunoassay in semi-quantitative mode with LC MS/MS – Serum

DRI TCA ST Assay Results	Negative by LC-MS/MS	< 50% of Cutoff concentration by LC-MS/MS (< 150 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (150-299 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (300-450 ng/mL)	High Positives (Greater than 50% above cutoff concentration (> 450 ng/mL)
Positive	0	2 [1]	0	31	25
Negative	1	34	24	0	0
% Negative Sample Agreement			97% or (59/61)		

% Positive Sample Agreement	100% or (56/56)
% Total Sample Agreement	98% or (115/117)

[1] Refer to Table 11 for discordant samples of serum.

[1]Table 11: Discordant Samples – Serum

Sample ID	Qualitative (mA/min) Result	Semi-Quantitative Observed concentration (ng/mL)	Final LC-MS/MS Value (ng/mL)
APP9489-2[2]	Negative	354 (Positive)	110
APP9474-2[3]	Positive	418 (Positive)	120

[2] Sample APP9489-2 contains Amitriptyline at 8.08 ng/mL, Imipramine at 18.35 ng/mL, Desipramine at 46.35 ng/mL, and Nortriptyline at 17 ng/mL by LC-MS/MS and cross-reacts at 100%, 158%, 120%, and 100% by immunoassay, respectively.

[3] Sample APP9474-2 contains Amitriptyline at 9.08 ng/mL, Imipramine at 19.9 ng/mL, Desipramine at 49.8 ng/mL, and Nortriptyline at 19.65 ng/mL by LC-MS/MS and cross-reacts at 100%, 158%, 120%, and 100% by immunoassay, respectively.

Note: In addition, both samples APP9489-2 and APP9474-2 were confirmed by LC-MS/MS to contain Carbamazepine and Carbamazepine Epoxide with concentrations (4275 ng/mL & 5285 ng/mL) and (595 ng/mL & 955 ng/mL), respectively.

Table 12: Stratified data comparing immunoassay in qualitative mode with LC-MS/MS – Urine

DRI TCA ST Assay Results	Negative by LC-MS/MS	< 50% of Cutoff concentration by LC-MS/MS (< 150 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (150-299 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (300-450 ng/mL)	High Positives (Greater than 50% above cutoff concentration (> 450 ng/mL)
Positive	0	0	2[4]	7	42
Negative	22	11	15	1[4]	0
% Negative Sample Agreement			96% or (48/50)		
% Positive Sample Agreement			98% or (49/50)		
% Total Sample Agreement			97% or (97/100)		

[4] Refer to Table 14 for discordant samples of urine.

Table 13: Stratified data comparing immunoassay in semi-quantitative mode with LC-MS/MS – Urine

DRI TCA ST Assay Results	Negative by LC-MS/MS	< 50% of Cutoff concentration by LC-MS/MS (< 150 ng/mL)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration as determined by LC-MS/MS) (150-299 ng/mL)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration as determined by LC-MS/MS) (300-450 ng/mL)	High Positives (Greater than 50% above cutoff concentration (> 450 ng/mL)
Positive	0	0	2[4]	7	42
Negative	22	11	15	1[4]	0
% Negative Sample Agreement			96% or (48/50)		
% Positive Sample Agreement			98% or (49/50)		
% Total Sample Agreement			97% or (97/100)		

[4] Refer to Table 14 for discordant samples of urine.

[4]Table 14: Discordant Samples – Urine

Sample ID	Qualitative (mA/min) Result	Semi-Quantitative Observed concentration (ng/mL)	LC-MS/MS Value (ng/mL)
APP6058-2[5]	Positive	347 (Positive)	285
APP6060-2[6]	Positive	375 (Positive)	230
APP6056-2[7]	Negative	267 (Negative)	372

[5] Sample APP6058-2 contains Amitriptyline at 146 ng/mL and Nortriptyline at 139 ng/mL by LC-MS/MS and cross-reacts at 100% and 100% by immunoassay, respectively.

[6] Sample APP6060-2 contains Amitriptyline at 114 ng/mL, Nordoxepin at 3.05 ng/mL, and Nortriptyline at 115 ng/mL by LC-MS/MS and cross-reacts at 100%, 17.1% and 100% by immunoassay, respectively.

[7] Sample APP6056-2 contains Amitriptyline at 186 ng/ mL and Nortriptyline at 186 ng/mL by LC-MS/MS and cross-reacts at 100% and 100% by immunoassay, respectively.

e) Matrix equivalency

Matrix Equivalency is performed in accordance with CLSI Guideline *CLSI-EP35.*

Two (2) replicates of 50 patient samples of K2 EDTA Plasma, K3 EDTA plasma, Lithium Heparin Plasma, Sodium Citrate Plasma, Potassium Oxalate Plasma and 45 patient samples of Sodium Heparin Plasma were run in both qualitative and semi-quantitative modes to demonstrate Nortriptyline concentrations obtained in different test plasma matrices with different anticoagulants are equivalent to those measured in the primary or control matrix, serum. The results of the matrix equivalency study are presented in the table below.

Table 15: Matrix Equivalency results in qualitative and semi-quantitative mode

Serum Matrix		Qualitative		Semi-quantitative	
		Positive	Negative	Positive	Negative
K2 EDTA Plasma	Positive	25	0	25	0
	Negative	0	25	0	25
K3 EDTA plasma	Positive	25	0	25	0
	Negative	0	25	0	25
Lithium Heparin Plasma	Positive	25	0	25	0
	Negative	0	25	0	25
Sodium Citrate Plasma	Positive	25	0	25	0
	Negative	0	25	0	25
Potassium Oxalate Plasma	Positive	25	0	25	0
	Negative	0	25	0	25
Sodium Heparin Plasma	Positive	25	0	25	0
	Negative	0	20	0	20

f) Specificity

Cross-Reactivity to structurally related compounds:
The cross-reactivity of Tricyclic compounds and other structurally related compounds were evaluated by adding known amount of each compound into drug-free serum and drug-free urine at concentrations indicated. Serum and urine samples were run separately.

Table 16: Cross Reactivity to structurally related compounds in Serum
(TCA drugs and Metabolites)

Structurally Related Compounds (TCA drugs and Metabolites)	Serum		
	Tested Concentrations (ng/mL)	Positive/ Negative	Cross-Reactivity (%)
2-Hydroxy Imipramine	1,300	Positive	23.1
7-Hydroxy Quetiapine	100,000	Negative	< 0.3
7-Hydroxy Amoxapine	100,000	Negative	< 0.3
8-Hydroxy Amoxapine	100,000	Negative	< 0.3
Amitriptyline	300	Positive	100
Amoxapine	125,000	Positive	0.2
10-Hydroxyamitriptyline	1,000	Positive	30
Clomipramine	300	Positive	100
Desipramine	250	Positive	120
Dosulepin	475	Positive	63.2
Doxepin	600	Positive	50
Imipramine	190	Positive	157.9
Lofepramine	430	Positive	69.8
N-Desmethyltrimipramine	350	Positive	85.7
Norclomipramine	375	Positive	80
Nordoxepin	1,750	Positive	17.1
Nortriptyline	300	Positive	100
Opipramol	350	Positive	85.7
Protriptyline	450	Positive	66.7
Quetiapine Fumarate	50,000	Positive	0.6
Trimipramine	390	Positive	76.9

Table 17: Cross Reactivity to structurally related compounds in Urine
(TCA drugs and Metabolites)

Structurally Related Compounds (TCA drugs and Metabolites)	Urine		
	Tested Concentrations (ng/mL)	Positive/ Negative	Cross-Reactivity (%)
2-Hydroxy Imipramine	1,000	Positive	30
7-Hydroxy Quetiapine	100,000	Negative	< 0.3
7-Hydroxy Amoxapine	100,000	Negative	< 0.3
8-Hydroxy Amoxapine	100,000	Negative	< 0.3
Amitriptyline	300	Positive	100
Amoxapine	100,000	Positive	0.3

Structurally Related Compounds (TCA drugs and Metabolites)	Urine		
	Tested Concentrations (ng/mL)	Positive/ Negative	Cross-Reactivity (%)
10-Hydroxyamitriptyline	700	Positive	42.9
Clomipramine	350	Positive	85.7
Desipramine	250	Positive	120
Dosulepin	425	Positive	70.6
Doxepin	550	Positive	54.5
Imipramine	220	Positive	136.4
Lofepramine	460	Positive	65.2
N-Desmethyltrimipramine	325	Positive	92.3
Norclomipramine	450	Positive	66.7
Nordoxepin	1750	Positive	17.1
Nortriptyline	300	Positive	100
Opipramol	350	Positive	85.7
Protriptyline	400	Positive	75
Quetiapine Fumarate	45,000	Positive	0.7
Trimipramine	400	Positive	75

Table 18: Cross Reactivity to structurally related compounds in Serum
(Other compounds)

Other Structurally Related Compounds	Serum		
	Tested Concentrations (ng/mL)	Positive/ Negative	Cross-Reactivity (%)
Alimemazine	12,000	Positive	2.5
Blonanserin	100,000	Negative	< 0.3
Chlorpromazine	525	Positive	57.1
Clozapine	100,000	Negative	< 0.3
Cyclobenzaprine	450	Positive	66.7
Desloratadine	100,000	Negative	< 0.3
Diphenhydramine	60,000	Positive	0.5
Fluoxetine	100,000	Negative	< 0.3
Fluphenazine	2,000	Positive	15
Haloperidol	100,000	Negative	< 0.3
Loratadine	100,000	Negative	< 0.3
Loxapine	100,000	Negative	< 0.3
Maprotiline	100,000	Positive	0.3
Mianserin	100,000	Negative	< 0.3
Mirtazapine	100,000	Negative	< 0.3
N-Desmethylclozapine	100,000	Negative	< 0.3
Nefazodone	100,000	Negative	< 0.3
N-Desmethylmirtazapine	100,000	Negative	< 0.3
Normaprotiline	100,000	Positive	0.3
Olanzapine	100,000	Negative	< 0.3
Paroxetine	100,000	Negative	< 0.3

Perphenazine	450	Positive	66.7
Phenoxybenzamine	100,000	Negative	< 0.3
Promazine	400	Positive	75
Promethazine	20,000	Positive	1.5
Risperidone	100,000	Negative	< 0.3
Sertraline	100,000	Negative	< 0.3
Thioridazine	6,000	Positive	5
Thiothixene	100,000	Negative	< 0.3
Tianeptine	100,000	Negative	< 0.3
Trazodone	100,000	Negative	< 0.3
Ziprasidone	100,000	Negative	< 0.3

Table 19: Cross Reactivity to structurally related compounds in Urine
(Other compounds)

Other Structurally Related Compounds	Urine		
	Tested Concentrations (ng/mL)	Positive/ Negative	Cross-Reactivity (%)
Alimemazine	12,000	Positive	2.5
Blonanserin	100,000	Negative	< 0.3
Chlorpromazine	600	Positive	50
Clozapine	100,000	Negative	< 0.3
Cyclobenzaprine	500	Positive	60
Desloratadine	100,000	Negative	< 0.3
Diphenhydramine	30,000	Positive	1
Fluoxetine	100,000	Negative	< 0.3
Fluphenazine	2,000	Positive	15
Haloperidol	100,000	Negative	< 0.3
Loratadine	100,000	Negative	< 0.3
Loxapine	100,000	Positive	0.3
Maprotiline	100,000	Positive	0.3
Mianserin	100,000	Negative	< 0.3
Mirtazapine	100,000	Negative	< 0.3
N-Desmethylclozapine	100,000	Negative	< 0.3
Nefazodone	100,000	Negative	< 0.3
N-Desmethylmirtazapine	100,000	Negative	< 0.3
Normaprotiline	100,000	Positive	0.3
Olanzapine	100,000	Negative	< 0.3
Paroxetine	100,000	Negative	< 0.3
Perphenazine	650	Positive	46.2
Phenoxybenzamine	100,000	Negative	< 0.3
Promazine	410	Positive	73.2
Promethazine	15,000	Positive	2
Risperidone	100,000	Negative	< 0.3
Sertraline	100,000	Negative	< 0.3

Other Structurally Related Compounds	Urine		
	Tested Concentrations (ng/mL)	Positive/ Negative	Cross-Reactivity (%)
Thioridazine	4,000	Positive	7.5
Thiothixene	100,000	Negative	< 0.3
Tianeptine	90,000	Positive	0.33
Trazodone	100,000	Negative	< 0.3
Ziprasidone	100,000	Negative	< 0.3

Cross-reactivity to structurally unrelated compounds:
Structurally unrelated compounds and/or concurrently used drugs were spiked at the concentration listed below into low and high controls (225 and 375 ng/mL) in serum and urine. Low control as negative and high control as positive indicate that all the compounds evaluated exhibited minimal cross-reactivity at the concentration tested. Serum and urine samples were run separately. The study results are presented in the table below:

Table 20: Cross Reactivity to structurally unrelated compounds in Serum

Structurally Unrelated Compounds	Serum		
	Tested Concentrations (ng/mL)	Low Control -25% of cutoff (225 ng/mL)	High Control +25% of cutoff (375 ng/mL)
11-nor-Δ9-THC-COOH	100,000	Negative	Positive
6-Acetyl morphine	75,000	Negative	Positive
Acetaminophen	100,000	Negative	Positive
Acetylsalicylic acid	100,000	Negative	Positive
Amisulpride	100,000	Negative	Positive
Amoxicillin	100,000	Negative	Positive
Amphetamine	100,000	Negative	Positive
Benztropine Methane Sulfonate	3,000	Negative	Positive
Benzoylecgonine	100,000	Negative	Positive
Brompheniramine	3,000	Negative	Positive
Caffeine	100,000	Negative	Positive
Carbamazepine	3,000	Negative	Positive
Carbamazepine Epoxide	10,000	Negative	Positive
Chloroquine phosphate	100,000	Negative	Positive
Cimetidine	100,000	Negative	Positive
Cocaine	75,000	Negative	Positive
Codeine	100,000	Negative	Positive
Dextromethorphan	100,000	Negative	Positive
Diacetylmorphine (Heroin)	100,000	Negative	Positive
Diazepam	100,000	Negative	Positive
Digoxin	100,000	Negative	Positive
Dihydrocodeine	100,000	Negative	Positive
EDDP Perchlorate	100,000	Negative	Positive

Structurally Unrelated Compounds	Serum		
	Tested Concentrations (ng/mL)	Low Control -25% of cutoff (225 ng/mL)	High Control +25% of cutoff (375 ng/mL)
EMDP-HCL	100,000	Negative	Positive
Fentanyl	25,000	Negative	Positive
Glutethimide	100,000	Negative	Positive
Hydrocodone	100,000	Negative	Positive
Hydrocortisone	100,000	Negative	Positive
Hydromorphone	100,000	Negative	Positive
Hydroxyzine	3,000	Negative	Positive
Ibuprofen	100,000	Negative	Positive
Levorphanol-D3	100,000	Negative	Positive
Levothyroxine	100,000	Negative	Positive
Meperidine	25,000	Negative	Positive
Methadone	75,000	Negative	Positive
Methamphetamine	100,000	Negative	Positive
Methaqualone	500	Negative	Positive
Methsuximide	75,000	Negative	Positive
Methylphenidate	100,000	Negative	Positive
Morphine	100,000	Negative	Positive
Morphine-3β-glucuronide	100,000	Negative	Positive
Morphine-6β-glucuronide	100,000	Negative	Positive
Nalbuphine	100,000	Negative	Positive
Nalorphine	100,000	Negative	Positive
Naloxone	100,000	Negative	Positive
Naltrexone	100,000	Negative	Positive
Naproxen	100,000	Negative	Positive
Norcodeine	100,000	Negative	Positive
Nordiazepam	100,000	Negative	Positive
Norethindrone	100,000	Negative	Positive
Norhydrocodone	100,000	Negative	Positive
Noroxycodone	100,000	Negative	Positive
Noroxymorphone	100,000	Negative	Positive
Norpropoxyphene	75,000	Negative	Positive
Oxaprozin	100,000	Negative	Positive
Oxazepam	100,000	Negative	Positive
Oxycodone	100,000	Negative	Positive
Oxymorphone	100,000	Negative	Positive
PCP	50,000	Negative	Positive
Phenobarbital	100,000	Negative	Positive
Phenytoin	100,000	Negative	Positive
Primidone	100,000	Negative	Positive
Procyclidine	100,000	Negative	Positive
Propoxyphene	100,000	Negative	Positive
Secobarbital	100,000	Negative	Positive

Structurally Unrelated Compounds	Serum		
	Tested Concentrations (ng/mL)	Low Control -25% of cutoff (225 ng/mL)	High Control +25% of cutoff (375 ng/mL)
Tapentadol	100,000	Negative	Positive
Temazepam	100,000	Negative	Positive
Triprolidine	100,000	Negative	Positive
Valproic Acid	100,000	Negative	Positive
Venlafaxine	100,000	Negative	Positive
Verapamil	100,000	Negative	Positive

Table 21: Cross Reactivity to structurally unrelated compounds in Urine

Structurally Unrelated Compounds	Urine		
	Tested Concentrations (ng/mL)	Low Control -25% of cutoff (225 ng/mL)	High Control +25% of cutoff (375 ng/mL)
11-nor-Δ9-THC-COOH	100,000	Negative	Positive
6-Acetyl morphine	100,000	Negative	Positive
Acetaminophen	100,000	Negative	Positive
Acetylsalicylic acid	100,000	Negative	Positive
Amisulpride	100,000	Negative	Positive
Amoxicillin	100,000	Negative	Positive
Amphetamine	100,000	Negative	Positive
Benztropine Methane Sulfonate	7,000	Negative	Positive
Benzoylecgonine	100,000	Negative	Positive
Brompheniramine	5,000	Negative	Positive
Caffeine	100,000	Negative	Positive
Carbamazepine	5,000	Negative	Positive
Carbamazepine Epoxide	30,000	Negative	Positive
Chloroquine phosphate	100,000	Negative	Positive
Cimetidine	100,000	Negative	Positive
Cocaine	100,000	Negative	Positive
Codeine	100,000	Negative	Positive
Dextromethorphan	100,000	Negative	Positive
Diacetylmorphine (Heroin)	100,000	Negative	Positive
Diazepam	100,000	Negative	Positive
Digoxin	100,000	Negative	Positive
Dihydrocodeine	100,000	Negative	Positive
EDDP Perchlorate	100,000	Negative	Positive
EMDP-HCL	100,000	Negative	Positive
Fentanyl	25,000	Negative	Positive
Glutethimide	100,000	Negative	Positive
Hydrocodone	100,000	Negative	Positive
Hydrocortisone	100,000	Negative	Positive
Hydromorphone	100,000	Negative	Positive
Hydroxyzine	5,000	Negative	Positive
Ibuprofen	100,000	Negative	Positive
Levorphanol-D3	100,000	Negative	Positive

Structurally Unrelated Compounds	Urine		
	Tested Concentrations (ng/mL)	Low Control -25% of cutoff (225 ng/mL)	High Control +25% of cutoff (375 ng/mL)
Levothyroxine	100,000	Negative	Positive
Meperidine	25,000	Negative	Positive
Methadone	75,000	Negative	Positive
Methamphetamine	100,000	Negative	Positive
Methaqualone	1,000	Negative	Positive
Methsuximide	75,000	Negative	Positive
Methylphenidate	100,000	Negative	Positive
Morphine	100,000	Negative	Positive
Morphine-3β-glucuronide	100,000	Negative	Positive
Morphine-6β-glucuronide	100,000	Negative	Positive
Nalbuphine	100,000	Negative	Positive
Nalorphine	100,000	Negative	Positive
Naloxone	100,000	Negative	Positive
Naltrexone	100,000	Negative	Positive
Naproxen	100,000	Negative	Positive
Norcodeine	100,000	Negative	Positive
Nordiazepam	100,000	Negative	Positive
Norethindrone	100,000	Negative	Positive
Norhydrocodone	75,000	Negative	Positive
Noroxycodone	100,000	Negative	Positive
Noroxymorphone	100,000	Negative	Positive
Norpropoxyphene	75,000	Negative	Positive
Oxaprozin	100,000	Negative	Positive
Oxazepam	100,000	Negative	Positive
Oxycodone	100,000	Negative	Positive
Oxymorphone	100,000	Negative	Positive
PCP	75,000	Negative	Positive
Phenobarbital	100,000	Negative	Positive
Phenytoin	100,000	Negative	Positive
Primidone	100,000	Negative	Positive
Procyclidine	100,000	Negative	Positive
Propoxyphene	100,000	Negative	Positive
Secobarbital	100,000	Negative	Positive
Tapentadol	100,000	Negative	Positive
Temazepam	100,000	Negative	Positive
Triprolidine	100,000	Negative	Positive
Valproic Acid	100,000	Negative	Positive
Venlafaxine	100,000	Negative	Positive
Verapamil	100,000	Negative	Positive

g) Interference

The interference study is performed per the *CLSI EP07. 3rd edition.* The potential interference of endogenous, exogenous, physiological substances, and pH on the recovery of nortriptyline using DRI Tricyclics Serum Tox Assay was assessed. Potentially interfering substances were spiked into the low control, 225ng/mL (-25% of the cutoff concentration of 300 ng/mL) and high controls, 375 ng/mL (+25% of the cutoff concentration of 300 ng/mL). Serum and urine samples were run separately.

As shown in the tables below, the controls were detected accurately. In the presence of the compounds listed below, the controls were detected accurately, indicating that these compounds did not show interference in the assay.

Table 22: Results of Interfering Substances Testing – Serum

Compounds	Tested Concentrations (mg/dL)	Low Control -25% of cutoff (225 ng/mL)	High Control +25% of cutoff (375 ng/mL)
Bilirubin (Conjugated)	40	Negative	Positive
Bilirubin (Unconjugated)	40	Negative	Positive
Hemoglobin	1000	Negative	Positive
Albumin	7500	Negative	Positive
γ-globulin	5000	Negative	Positive
Rh Factor	1300 IU	Negative	Positive
Triglycerides	1500	Negative	Positive
Cholesterol	1400	Negative	Positive

Table 23: Results of Interfering Substances Testing – Urine

Compounds	Tested Concentrations (mg/dL)	Low Control -25% of cutoff (225 ng/mL)	High Control +25% of cutoff (375 ng/mL)
Acetone	500	Negative	Positive
Ascorbic Acid	150	Negative	Positive
Caffeine	5	Negative	Positive
Creatinine	400	Negative	Positive
Ethanol	1000	Negative	Positive
Galactose	5	Negative	Positive
Glucose	1000	Negative	Positive
Hemoglobin	150	Negative	Positive
Human Serum Albumin (HSA)	200	Negative	Positive
Oxalic acid	50	Negative	Positive
Riboflavin	3	Negative	Positive
Sodium Chloride	1000	Negative	Positive
Urea	1000	Negative	Positive

pH interference for urine only:
The intermediate drug stock at 10,000 ng/mL was used to spike the pH adjusted drug free urine samples to make low control, 225 ng/mL and high control, 375 ng/mL with pH range from 3 to 11. The results are presented in the table below:

Table 24: pH interference study results for urine

pH	Low Control -25% of cutoff (225 ng/mL)	High Control +25% of cutoff (375 ng/mL)
3	Negative	Positive
4	Negative	Positive
5	Negative	Positive
6	Negative	Positive
7	Negative	Positive
8	Negative	Positive
9	Negative	Positive
10	Negative	Positive
11	Negative	Positive

There is no need to test pH as interfering factor for serum because blood pH range is very narrow. Any continuous pH < 7.3 and > 7.8 are lethal. Varied urine pH is to support in case of sample adulteration.

Specific gravity for urine only:
The specific gravity of multiple negative urine samples from different donors were tested on available clinical chemistry analyzer to select ten (10) negative urine samples with specific gravity range from 1.004 to 1.030.

The selected negative urine samples with a specific gravity range from 1.004 to 1.030 were spiked at control levels of 225 ng/mL and 375ng/mL using intermediate drug stock at 10,000 ng/mL.

Table 25: Specific gravity study results for urine

Specific Gravity	Low Control -25% of cutoff (225 ng/mL)	High Control +25% of cutoff (375 ng/mL)
1.004	Negative	Positive
1.006	Negative	Positive
1.008	Negative	Positive
1.010	Negative	Positive
1.011	Negative	Positive
1.012	Negative	Positive
1.013	Negative	Positive
1.016	Negative	Positive
1.022	Negative	Positive
1.030	Negative	Positive

H. Conclusion

The information supports a determination of substantial equivalence between DRI™ Tricyclics Serum Tox Assay (K213875) and the predicate device Tricyclic Serum Tox EIA Assay (K983268).