510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k102346

B. Purpose for Submission:

New device

C. Measurand:

Digoxin

D. Type of Test:

Quantitative enzyme immunoassay

E. Applicant:

Randox Laboratories Limited

F. Proprietary and Established Names:

Randox Digoxin Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.3320

2. Classification:

   Class II

3. Product code:

   KXT

4. Panel:

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below

2. Indication(s) for use:

   The ***Randox*** Laboratories Digoxin Assay is an in vitro diagnostic test for the quantitative determination of Digoxin in human serum or plasma.

   Quantitative measurements are used in the diagnosis and treatment of Digoxin overdose and in monitoring levels of Digoxin to ensure appropriate therapy.

   This Device has been developed for the ADVIA system and is intended for prescription use only.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   For use on the ADVIA 1650 system

I. Device Description:

The assay consists of ready-to-use liquid reagents. Reagent 1, the antibody buffer, contains 8 mL of mouse monoclonal anti-digoxin antibody (0.1%), and sodium azide (0.09% w/v). Reagent 2, the latex reagent, contains 6 mL of digoxin-coated latex beads and sodium azide (0.09% w/v). The calibrators and controls are sold separately. The kit contains 2 vials of each reagent.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Siemens *Emit* 2000 Digoxin Assay

2. Predicate 510(k) number(s):

   k011920

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Quantitative analysis of Digoxin in human serum or plasma	Same
Sample Type	Human serum or plasma (Li-Heparin)	Same
Reagent Type	Liquid ready-to-use	Same

Differences		
Item	Device	Predicate
Instrumentation	Advia 1650	Various chemistry analyzers
Test Principle	Latex-enhanced immunoturbidimetric assay	Homogeneous enzyme immunoassay
Antibody Source	Mouse	Rabbit
Measuring Range	0.4 – 5.0 ng/mL	0.2 – 5.0 ng/mL

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

CLSI EP5-A2, Evaluation of Precision Performance of Qualitative Measurement Methods

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation

L. Test Principle:

The Randox Digoxin immunoturbidimetric assay for the determination of digoxin in human serum and plasma consists of various *in vitro* diagnostic test reagents. The immunoassay utilized in this test system is based upon the principle of measuring changes in scattered light. When digoxin is present in the sample, it competes with a digoxin-latex complex for binding with the anti-digoxin antibody and inhibits the formation of the agglutination complex. The rate of agglutination is inversely proportional to the sample concentration of digoxin. By monitoring the change in scattered light (absorbance at 694 nm) caused by varying degrees of agglutination, a concentration curve is obtained and the amount of digoxin can be calculated.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was evaluated following guideline EP5-A2. Precision samples were comprised of drug-free human serum spiked with pure digoxin. Two replicates of each sample were tested twice a day on 20 separate days, yielding 80 replicates total over 40 runs. Each precision study was completed on 2 separate Advia 1650

instruments (numbers 505 and 786).

Precision Results Advia 1650 System Number 505

Sample	Units = ng/mL				
	Mean Digoxin Conc.	Within-run CV (%)	Total CV (%)	No. Observ.	No. Days
Sample 1	0.52	9.0	10.1	80	20
Sample 2	0.84	5.3	6.3	80	20
Sample 3	1.58	1.7	3.0	80	20
Sample 4	2.95	0.9	2.7	80	20
Sample 5	4.34	1.5	1.8	80	20

Precision Results Advia 1650 System Number 786

Sample	Units = ng/mL				
	Mean Digoxin Conc.	Within-run CV (%)	Total CV (%)	No. Observ.	No. Days
Sample 1	0.53	7.6	11.0	80	20
Sample 2	0.81	3.9	7.6	80	20
Sample 3	1.56	2.3	3.5	80	20
Sample 4	2.94	1.2	3.3	80	20
Sample 5	4.35	1.4	4.0	80	20

Within run precision was also evaluated using plasma samples in a 10-day study. Precision samples were comprised of drug-free human plasma spiked with pure digoxin. Two replicates of each sample were tested twice a day on 10 separate days, yielding 40 replicates total over 20 runs. Each sample was tested on 2 separate Advia 1650 instruments (numbers 505 and 786).

Precision Results Advia 1650 System Number 505

Sample	Units = ng/mL	
	Mean Digoxin Conc.	Within-run CV (%)
Sample 1	0.82	6.1
Sample 2	2.12	2.0
Sample 3	4.19	1.9

Precision Results Advia 1650 System Number 786

Sample	Units = ng/mL	
	Mean Digoxin Conc.	Within-run CV (%)
Sample 1	0.84	6.3
Sample 2	2.17	1.7
Sample 3	4.35	3.0

b. Linearity/assay reportable range:

Linearity was evaluated using drug-free human plasma with pure digoxin. High spiked pool samples were diluted using low pool samples to give a total of 9 dilutions, ranging from 0.41 ng/mL to 5.79 ng/mL. The samples were measured in triplicate on a single Advia 1650 instrument using one lot of reagent. Statistical evaluation gave the following results:

Level	Units = ng/mL		% Recovery
	Expected Conc.	Observed Conc.	
1	0.41	0.41	100
2	1.09	1.05	96.7
3	1.76	1.89	107.3
4	2.43	2.43	99.9
5	3.10	2.98	96.1
6	3.77	3.46	91.6
7	4.45	4.01	90.2
8	5.12	4.75	92.9
9	5.79	5.79	100

Plasma Linearity Study:

$y = 0.94x + 0.060$, $R^2 = 0.9912$

The reportable range of the assay is defined as the functional level of quantitation to the highest calibrator concentration. The stated reportable range in the device package insert is 0.4 ng/mL to 5.0 ng/mL

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

Traceability

The Randox Digoxin method is traceable to an internal standard manufactured using highly purified material. This internal standard is compared to USP material by comparison analysis using five equally spaced concentration intervals across the assay AMR, with triplicate analysis using only one lot of reagent. The sponsor specifies that individual recovery for each internal standard must be within ± 10% of the USP material concentration.

Value Assignment

Calibrators (previously cleared in k012318) are derived from a calibrator master lot, which is prepared form the internal standards and compared to the USP material for

value verification. Verification testing also uses both the calibrators being value assigned and previously assigned calibrators, and compares the values.

Controls (previously cleared in k012319) are value assigned using the both the master calibrators and the current assay calibrators.

The sponsor stated that calibrators and controls must have a % difference from the USP material of ≤ 5% difference (and any USP prepared dilutions must recover within 10% of their target concentration).

Stability

Calibrator and control stability was previously cleared in k012318 and k012319 respectively.

Calibration Interval

Stability across a 30 day calibration interval was assessed by calculating the percentage bias of the precision samples on each day from the result obtained for that precision samples from the calibration on the first day. The results support the product claim of a 7-day calibration interval.

d. Detection limit:

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined following guideline EP17-A.

For LoB determination, 2 replicates of the low calibrator were performed in 2 runs per day over 20 operator days, using 2 analyzers and one reagent lot – giving 80 determinations in total.

For LoD determination, 2 replicates of a low sample were performed in 2 runs per day over 20 operator days, using 2 analyzers and one reagent lot – giving 80 determinations in total. The low sample was made by spiking a drug-free human serum pool with pure digoxin.

For LoQ determination, ten replicates of each of six serum pools were run with two instruments over five days using one reagent lot – giving 120 determinations in total for each serum pool. The sponsor stated that the bias and standard deviation at the LoQ (0.4 ng/mL) was 0.00 and 0.44 respectively, and the total imprecision CV at 0.4 ng/mL was 11.0%.

Result Summary:

Based on the study result, the following detection limit claims were made:

LoB	LoD	LoQ
0.01 ng/mL	0.04 ng/mL	0.4 ng/mL

e. Analytical specificity:

Interference

Study Protocol:
The sponsor evaluated the effect of the interfering substances using two patient serum pools with spiked digoxin at approximately 0.8 ng/mL and 2.8 ng/mL. For each substance, multiple aliquots of each pool were spiked with the test substance up to the maximum level shown below. Samples were tested in duplicate and the results of the pools with interferent were compared to the pools without interferent, and % interference was calculated.

Result Summary:
Based on the sponsor-defined interference limit of ± 10%, the following claims were made:

- The below compounds at the indicated concentration do not cause significant interference with the assay.

Compound	Concentration up to
Bilirubin (unconjugated)	27.4 mg/dL
Bilirubin (conjugated)	22.2 mg/dL
Hemoglobin	1000 mg/dL
Lipemia (Intralipid)	798 mg/dL
Triglycerides	1000 mg/dL
Cholesterol	500 mg/dL
Gamma-globulin	5000 mg/dL
Protein (total)	12000 mg/dL
Protein (albumin)	6000 mg/dL
Rheumatoid Factor	700 IU/mL
Uric Acid	27.9 mg/dL

Cross-Reactivity

Study Protocol:
The sponsor evaluated cross-reactivity using two methods. First, endogenous and non-structurally related compounds were tested in the presence of 1 ng/mL digoxin, and the levels ion cross-reactant tested were at or above the maximum physiological or pharmacological concentration. A dilution series was prepared by dilution a serum pool containing a concentration of 1 ng/mL digoxin with the highest level of cross-reactant with the same pool without the cross-reactant (control), to yield five dilutions for testing each cross-reactant. The second study was conducted to test structurally related substances. The cross-reactants were evaluated in both digoxin-free serum and serum containing 2.34 ng/mL digoxin. Each cross-reactant was assayed in duplicate with the corresponding control.

Result Summary:
Based on a sponsor-defined cross reactivity of >10% difference from control, none of the tested substances at the indicated concentrations below will significantly cross

react with the proposed assay.

Category	Cross-reactant Tested	Concentration Tested
Endogenous Substance	Cortisol	10.0 µg/mL
	Estriol	10.0 µg/mL
	Prednisolone	10.0 µg/mL
	Prednisone	7.0 µg/mL
	Progesterone	5.0 µg/mL
	Testosterone	5.0 µg/mL
	Dehydroisandrosterone	50.0 µg/mL
Non-structurally Related Substance	Dexamethasone	25.0 µg/mL
	Furosemide	50.0 µg/mL
	Hydrochlorothiazide	75.0 µg/mL
	Lidocaine	100.0 µg/mL
	Propranolol	75.0 µg/mL
	Quinidine	100.0 µg/mL
	Secobarbitol	100.0 µg/mL
	Spironolactone	10.0 µg/mL
Structurally Related Substance	Deslanoside	2.5 ng/mL
	Digotoxin	25 ng/mL
	Digoxigenin	5 ng/mL
	Digitoxigenin	3.7 ng/mL
	Digitonin	25 ng/mL

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Study Protocol:
A total of 56 patient serum samples obtained from a vendor were used in the method comparison studies. Samples were assayed in duplicate and ranged from 0.56 ng/mL to 5.00 ng/mL. Regression analysis was performed between the Randox Digoxin method on the Advia 1650 system and the predicate Syva Emit Digoxin assay using the first of each duplicate measurement.

Result Summary:
Linear regression analysis yielded the equation $y = 0.97x – 0.22$, with an r value of 0.984.

b. Matrix comparison:

Study Protocol:
The matrix effect of Li-Heparin blood collection tubes on patient samples were evaluated. 40 matched patient serum and plasma samples ranging from 0.53 ng/mL

to 4.95 ng/mL were evaluated using one lot of reagent and analyzed in duplicate on one Advia 1650 system.

Results:
The linear regression analysis yielded the equation y = 1.03x – 0.060, with an r value of 0.991.

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):

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The package insert states that the Randox Digoxin Assay measures digoxin concentrations in human serum or plasma containing 0.40 – 5.0 ng/ml (0.51 – 6.4 nmol/l) digoxin and that the therapeutic range of digoxin serum concentrations is 0.9 – 2 ng/ml (1.2 – 2.6 nmol/l), citing the following references:

Lewis RP: Clinical use of serum digoxin concentrations. Am J Cardiol, 1992; 69: 97G–107G.

Jogestrand T, Edner M, Haverling M: Clinical value of serum digoxin assays in outpatients: Improvement by the standardization of blood sampling. Am Heart J 1989; 117(5): 1076-1083.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a

substantial equivalence decision.