510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:
k112161

B. Purpose for Submission:
New device

C. Measurand:
Myoglobin

D. Type of Test:
Quantitative fluorometric immunoassay

E. Applicant:
Radiometer Medical ApS

F. Proprietary and Established Names:
AQT90 Flex Myo Test
AQT90 FLEX Myo CAL cartridge
AQT90 FLEX LQC Multi-CHECK, Levels 1-3
AQT90 FLEX analyzer

G. Regulatory Information:

Device Name	Product Code	Classification	Regulation	Panel
AQT90 Flex Myo Test	DDR: Myoglobin immunological test	Class II	21 CFR § 866.5680	Immunology (82)
AQT90 FLEX Myo CAL cartridge	JIT: Calibrator.	Class II	21 CFR § 862.1150	Clinical Chemistry (75)
AQT90 FLEX LQC Multi-CHECK, Levels 1-3	JJY: Quality control material	Class I, reserved	21 CFR § 862.1660	Clinical Chemistry (75)
AQT90 FLEX	KHO: Fluorometer for clinical use.	Class I	21 CFR § 862.2560	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   See indications for use below.

2. Indication(s) for use:
   AQT90 FLEX analyzer is for *in vitro* diagnostic use. The instrument is an immunoassay instrument based on the quantitative determination of time-resolved fluorescence to estimate the concentrations of clinically relevant markers on whole-blood and plasma specimens to which a relevant anticoagulant has been added. It is intended for use in point-of-care and laboratory settings.

   AQT90 FLEX Myo Test is an *in vitro* diagnostic assay for the quantitative determination of myoglobin in EDTA or lithium-heparin whole blood or plasma specimens on the AQT90 FLEX analyzer in point of care and laboratory settings. It is indicated for use as an aid in the rapid diagnosis of heart disease, e.g. acute myocardial infarction.

   AQT90 FLEX Myo CAL cartridge is for *in vitro* diagnostic use. For calibration adjustment of the Myo Test, as indicated on the cartridge, on the AQT90 FLEX analyzer.

   AQT90 FLEX LQC Multi-CHECK, Levels 1-3, is for *in vitro* diagnostic use. For use with the AQT90 FLEX analyzer as a liquid quality control serum (LQC) to monitor the precision of laboratory testing procedures for the analytes listed on the specification insert.

3. Special conditions for use statement(s):
   For prescription use and point-of-care use.

4. Special instrument requirements:
   AQT90 FLEX analyzer.

I. Device Description:

Component	Description
Myoglobin Assay and Calibrator	The AQT90 FLEX Myo Test consists of ten Test cartridges (product name: AQT90 FLEX Myo Test; function: myoglobin assay reagents) and one Calibration cartridge (product name: AQT90 FLEX Myo CAL; function: calibrator). The Test cartridge contains Test cups whereas the Calibration adjustment cartridge contains both test cups and calibration adjustments cups. The components are listed below: • Mouse monoclonal anti-myoglobin capture and tracer antibodies, approximately 500 ng/cup. • Bovine serum albumin. Bovine γ-globulin. Mouse IgG as blocker substance for heterophilic antibody interference. • Tris-(hydroxymethyl)-aminomethane (TRIS) buffer • Sodium azide < 0.1%. • Calibrator Only: Dried reagents including native purified myoglobin (approx. 5 ng/cup).

Control Material	AQT90 FLEX LQC Multi-CHECK, Levels 1-3 is comprised of three levels of control material (80, 150 and 315 ng/mL) in human serum. Each human donor unit used to manufacture this control was tested using FDA-accepted methods and found nonreactive for Hepatitis B Surface Antigen (HBsAg), antibody to Hepatitis C (HCV) and antibody to HIV-1/HIV-2.
Analyzer	The AQT90 FLEX Analyzer is a cartridge-based immunoassay analyzer which requires the appropriate assay cartridge, the sample and assay buffer. The analyzer has a built-in system to determine the hematocrit value of the whole blood sample aspirated at the start of a test. This value is required to calculate an accurate analyte concentration in plasma. The hematocrit value is determined through a conductivity measurement at two frequencies. The reagent buffer (AQT90 FLEX Reagent Pack) for the analyzer can be purchased separately and is composed of two assay buffer pouches and a waste compartment packed in a cassette. The components of the buffer solution (pH 7.5) for are listed below: • 4-(2-hydroxyethyl)-l-piperazineethanesulfonic acid (HEPES) • Sodium chloride • Diazolidinylurea (Germal I I , bactericide), 1 g/L Propyl p-hydroxybenzoate, 0.2 g/L

J. Substantial Equivalence Information:

Predicate device name	Predicate 510(k) number
Fisher Diagnostics ARCHITECT Stat Myo Immunoassay	k042924
Fisher Diagnostics ARCHITECT STAT Myoglobin Calibrators A-F	k042924
Bio-Rad Laboratories Liquichek Cardiac Markers Plus Control LT	k050537
Abbott Architect iSystem	k042924

Comparison with predicate:

Myoglobin Assay		
Item	Proposed Device AQT90 FLEX Myo Test	Predicate Device ARCHITECT Stat Myo Immunoassay (k042924)
Indication for Use	AQT90 FLEX Myo Test is an *in vitro* diagnostic assay for the quantitative determination of myoglobin	Same
Matrix	Whole blood and plasma	Serum and plasma

Principle	Quantitative time-resolved fluorimetric one-step sandwich immunoassay	Quantitative chemiluminescent two-step sandwich immunoassay
Reportable Range	20 to 900 ng/mL	0 to 1,200 ng/mL
Analyzer	AQT90 FLEX	ARCHITECT i System
Analyzer		
Item	Proposed Device AQT90 FLEX	Predicate Device Abbott Architect iSystem (k042924)
Indication for Use	An immunoassay instrument based on the quantitative determination of time-resolved fluorescence to estimate the concentrations of clinically relevant markers on whole-blood and plasma specimens to which a relevant anticoagulant has been added. It is intended for use in point-of-care and laboratory settings.	An immunoassay instrument based on the quantitative determination of chemiluminescense to measure and quantify analyte concentration in plasma and serum.
Test Format	A completely closed, fully automated system, using cartridge-based immunoassays in disposable cups. Offers possibility of running 15 different parameters at a time and has a total capacity of 200 tests without replenishment.	The ARCHITECT i System is an open, fully-automated, immunoassay system allowing random and continuous access, and priority processing.
Specimen Identification	Samples are identified from the barcode on blood collection test tube. The code is read automatically by the analyzer.	Same
Specimen Sampling and Handling	Blood is drawn into normal vacuum test tubes. The blood collection tube is placed directly in the analyzer. A volume of sample is automatically drawn from the tube through its rubber seal. The still closed tube is discarded. The used immunoassay cup is discarded into a closed waste bin in the analyzer.	Blood is drawn into normal vacuum test tubes, which are centrifuged to obtain plasma. The blood collection tube is placed directly in the analyzer. A volume of sample is automatically drawn from the tube through its rubber seal. The still closed tube is discarded.
Calibration	The analyzer can be automatically calibrated by	Calibration must be carried out each time a new reagent lot

	means of calibration cartridges at the time of test cartridge lot change.	number is used. Calibration is run automatically.
Quality Control	AQT FLEX LQC Multi-CHECK, Levels 1, 2 and 3.	Architect STAT Controls, Levels 1, 2 and 3.

Calibrator		
Item	Proposed Device AQT90 FLEX Myo CAL Cartridge	Predicate Device ARCHITECT STAT Myoglobin Calibrators A-F (k042924)
Indication for Use	For calibration adjustment of the Myo Test.	Same
Constituents	Sixteen calibration adjustment cups, which contain dried reagents including native purified myoglobin.	Purified cardiac myoglobin in Tris buffer with stabilizers
Traceability	The calibration is traceable to Scripps M0725	Purified human cardiac myoglobin reference material (primary reference) is a purchased product which is prepared by taking human heart tissue and mixing it in a buffer solution. The primary calibrator is then run on a commercially available assay to determine its concentration.
Calibration adjustment interval	Once per lot of AQT90 FLEX Myo Test cartridges and as often as required.	Upon new assay reagent lot number
In-use stability	24 hours on-board	30 days at 2-8 °C
Storage temperature	2-8 °C	-10 °C

Control		
Item	Proposed Device AQT90 FLEX LQC Multi-CHECK, Levels 1-3	Predicate Device Liquichek Cardiac Markers Plus Control LT (k050537)
Indication for Use	For use with the AQT90 FLEX analyzer as a liquid quality control serum (LQC) to monitor the precision of laboratory testing procedures for the analytes listed	Same

	on the specification insert.	
Form	Same	Liquid
Matrix	Same Each human donor unit used to manufacture this control was tested using FDA-accepted methods and found nonreactive for Hepatitis B Surface Antigen (HBsAg), antibody to Hepatitis C (HCV) and antibody to HIV-1/HIV-2.	Human serum based
Traceability	Scripps M0725	N/A

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

CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods
CLSI EP6-A: Evaluation of die Linearity of Quantitative Measurement Procedures
CLSI EP07-A2: Interference Testing in Clinical Chemistry
CLSI EP09-A2: Method Comparison and Bias Estimation Using Patient Samples
CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation
CLSI C2S-A3: Defining, Establishing, and Verifying Reference Intervals in die Clinical Laboratory
CLSI I/LA30-A: Immunoassay Interference by Endogenous Antibodies
EN 13640: Stability Testing of In Vitro Diagnostic Reagents
ISO 14971: Medical Devices - Application of risk management to medical devices
ISO 15223-1: Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied

L. Test Principle:

The test format for the AQT90 FLEX is an immunoassay based on time-resolved fluorescence using an europium (Eu) chelate as the fluorescent label. The test receptacles (300 µL test cups) for each assay contain the biotinylated antibodies used for the capture of the analyte, a separating layer of glucosides, and the europium-chelate labeled antibodies used to trace the captured proteins. The sample or diluted sample is added to the test cup together with assay buffer. The cup is then incubated to allow formation of the immuno-complex and washed to remove unbound antibodies and sample materials. Finally, the cup is exposed to excitation light and after a delay, the emitted light is measured by single photon counting; this measurement cycle is repeated up to 3,300 times. The total count (the response) is then compared to a calibration curve to obtain a quantitative measurement of the analyte's concentration in the sample.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

a. Precision/Reproducibility:

The precision of the AQT90 Flex Myo Test on the AQT90 FLEX analyzer was evaluated using whole blood and plasma samples at three point of care settings (POC) based upon the CLSI EP5-A2 guideline. Fresh native or antigen spiked lithium-heparin whole blood samples were tested in five replicates in five separate runs within three hours at three POC sites (one instrument, one test kit lot per site). Five levels of frozen native or antigen spiked lithium-heparin plasma were tested in duplicate in two separate runs for twenty days at three POC sites (one instrument, three test kit lots per site). The results are summarized in the following tables.

Summary of Whole Blood Precision Study Results

Site	Mean (ng/mL)	N	Within Run Precision		Total Precision	
			SD	%CV	SD	%CV
1	40	25	1.4	3.6	1.4	3.6
	107	25	4.0	3.7	4.0	3.7
	175	25	5.8	3.3	6.0	3.4
	293	25	8.1	2.8	8.1	2.8
	585	25	18.0	3.1	17.9	3.1
2	28	25	0.7	2.4	0.7	2.6
	99	25	3.1	3.1	3.1	3.1
	160	25	3.3	2.0	3.3	2.0
	303	25	7.0	2.3	7.0	2.3
	572	25	13.3	2.3	13.3	2.3
3	37	25	0.8	2.2	0.8	2.2
	132	25	3.1	2.3	3.1	2.3
	155	25	4.3	2.8	4.7	3.0
	298	25	6.1	2.1	7.4	2.5
	587	25	15.3	2.6	15.3	2.6

Summary of Plasma Precision Study Results

Site	Mean (ng/mL)	N	Within Run Precision		Total Precision	
			SD	%CV	SD	%CV
1	42	100	1.0	2.3	1.9	4.4
	95	100	1.7	1.8	4.0	4.2
	198	100	4.3	2.2	6.3	3.2
	378	100	7.9	2.1	12.0	3.2
	706	100	19.0	2.7	29.5	4.2
2	43	82	0.6	1.5	2.2	5.2
	98	84	2.1	2.1	5.0	5.1
	205	84	3.9	1.9	8.5	4.1
	389	84	13.5	3.5	18.3	4.7
	722	84	16.9	2.3	36.8	5.1
3	44	80	0.9	2.1	2.0	4.6
	99	80	2.5	2.5	5.3	5.3
	205	80	4.4	2.1	8.9	4.3

	389	80	6.6	1.7	16.1	4.1
	727	80	17.4	2.4	35.0	4.8

b. *Linearity/assay reportable range:*
The linearity of the AQT90 FLEX Myo Test was evaluated on the AQT90 FLEX analyzer by diluting two spiked plasma and whole blood samples with various levels of diluent to obtain twelve levels for each matrix. The plasma samples had values ranging from 0.04 to 1108 ng/mL while the blood samples ranged from 0.14 to 1766 ng/mL. The linear regression correlation is y= 0.98x – 4.21 for plasma samples and y= 1.00 + 0.36 for whole blood samples. The data provided support the sponsor's claims that the reportable range of this assay is 20 to 900 ng/mL.

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

Traceability and Value Assignment
The antigen material, M0725 by Scripps, has been used to establish the metrological traceability chain of the AQT90 FLEX Myo assay because there is no international conventional calibrator for myoglobin. The nominal concentration of the reference material at each concentration level has been defined by weighing the fraction of the reference material and the buffer solution.

This material has been used to assign values for the manufacturer's working calibrator with the manufacturer's selected measurement procedure. In summary, the reference material (eight concentration points) and the calibrator material (eight concentration points) have been measured with two instruments, two reagent batches, over three days and with four replicate measurements. Altogether this yields a total of 48 individual results produced by a known amount of specific protein from all reference and calibrator points.

The success of the value assignment and commutability of the two materials have been estimated by measuring patient samples against the standard curves of these two materials. The regression of averages of these concentrations was evaluated. The slope was not statistically significantly different from the expected value of 1, and the calculated intercept was not statistically different from the expected value of 0. This confirms the commutability of the materials.

The traceability chain from the manufacturer's working calibrator to the product calibrator is performed for all AQT90 FLEX Myo batches using three instruments, two runs and five replicate measurements. The value assignment from the working calibrator to the product calibrator and generation of the factory standard curve are done with total of 30 individual results of the manufacturer's working calibrator.

Control material was purchased from commercial available sources. The value assignment for each Multi-CHECK level includes two AQT90 FLEX Myo Test reagent lots, three AQT90 FLEX analyzers, two test days and five measurement replicates per control level for a total of 60 measurements per control level.

Stability

AQT90 FLEX Myo Test and Calibration Cartridges: The shelf life stability studies were performed on three product lots of test and calibration cartridges. The product was kept at 2 -8 °C and tested at different time points during ten months on the AQT90 FLEX analyzer. On-board stability was studied with three product lots of test and calibration cartridges stored at 2 -8 °C, placed at 32°C and tested at indicated time points (zero, six and eight months) on the AQT90 FLEX analyzer. Based on the results, the sponsor claims that test and calibration cartridges are claimed to have a shelf stability of 8 months at 2 to 8 °C, while the on-board stability is 16 days at 32 °C for the test cartridge and 48 hours at 32 °C for the calibration cartridge.

AQT90 FLEX LQC Multi-Check: Shelf life stability studies were performed on three lots. The product was kept at -18 to -26 °C, placed at 2 to 8 °C and tested at different time points up to fifteen months on the AQT90 FLEX analyzer. On-board stability was studied with three product lots placed at either 8 °C or 32 °C and tested at different time points up to fifteen hours on the AQT90 FLEX analyzer. Based on the results, the sponsor claims a shelf stability of 12 months at -18 to -26 °C, while the on-board stability is 4 days unopened at 2 to 8 °C or 2 hours opened at room temperature.

AQT90 FLEX Reagent Pack: Shelf life stability studies were performed on three product lots. The product was kept at 32 °C and tested at different time points up to fifteen months on the AQT90 FLEX analyzer. On-board stability was studied with one product lot placed at 32 to 40 °C and tested at different time points on the AQT90 FLEX analyzer. Based on the results, the sponsor claims a shelf stability of 5 months at 2 to 32 °C, while the on-board stability is 25 days at room temperature.

d. Detection limit:

The Limit of Blank (LoB) determination was based on 64 replicate measurements of AQT Assay Buffer on a single AQT90 FLEX instrument according to CLSI EP 17-A. The Limit of Blank as determined by the upper 95th percentile is 0.037 ng/mL. The Limit of Blank claim in the package insert is 0.5 ng/mL.

Limit of Detection (LoD) determination was based on 20 replicate measurements of four low samples on an AQT90 FLEX instrument. The LoD determination was calculated by using the determined LoB value and pooled SD values from LoD samples. The Limit of Detection was determined to be 0.2 ng/mL for BSA-buffer supplemented with red blood cells. The Limit of Detection claim in the package insert is 1.0 ng/mL.

Limit of Quantitation (LoQ) determination was based on 30 replicate measurements of four low samples on two AQT90 FLEX instruments. The Limit of Quantitation was determined to be 0.6 ng/mL for BSA-buffer supplemented with red blood cells. The Limit of Quantitation claim in the package insert is 1.0 ng/mL.

The AQT90 FLEX Myo Test has a reportable range of 20-900 ng/mL.

e. Analytical specificity:
Interference studies were designed according to the CLSI EP7-A guideline. Two levels of human plasma (100 and 500 ng/mL) were spiked with known interference substances (51 drugs, 3 solvents and 13 endogenous compounds) and samples were analyzed in five replicates on the AQT90 FLEX analyzer. No significant interference, defined as recovery within ± 10% compared to reference without any interfering substance, was observed with the assay. Specifically, no interference was observed with unconjugated Bilirubin up to 270 mg/L (27.0 mg/dL), conjugated Bilirubin up to 270 mg/L (27.0 mg/dL), Hemoglobin up to 10 g/L (1000 mg/dL) and triglycerides up to 15 g/L (1500 mg/dL).

The potential interference of thirteen endogenous compounds was evaluated in whole blood samples. Similar to the results observed with plasma samples, no interference was observed with unconjugated Bilirubin up to 270 mg/L (27.0 mg/dL),conjugated Bilirubin up to 270 mg/L (27.0 mg/dL), Hemoglobin up to 10 g/L (1000 mg/dL), and triglycerides up to 15 g/L (1500 mg/dL).

No hook effect was observed for plasma samples up to 1,950 ng/mL and up to 6,500 ng/mL with whole blood samples, which are outside of the reportable range of 20 to 900 ng/mL.

Potential positive interference from human anti-mouse antibody (HAMA) and Rheumatoid factor (RF) was evaluated (five replicate measurements) using seventeen human plasma samples containing HAMA and/or RF. The sponsor claims no positive interference from human anti-mouse antibody (HAMA) and Rheumatoid factor (RF).

Possible negative interference from HAMA and RF was evaluated by comparing the results of seventeen human HAMA and/or RF plasma samples spiked with myoglobin to reference sample (HAMA/RF negative) containing the same amount of myoglobin. No negative interference for the assay was observed, which was defined as recovery within ± 10% of human HAMA/RF positive samples compared to a reference sample (HAMA/RF negative). The sponsor claims no negative interference from human anti-mouse antibody (HAMA) and Rheumatoid factor (RF).

f. Assay cut-off:
Not applicable

2. Comparison studies:

a. Method comparison with predicate device:
Paired human whole blood and lithium-heparin plasma samples were collected at four hospitals and measurements were obtained using the AQT90 FLEX Myo Test run on the AQT90 FLEX analyzer. Frozen plasma samples were sent to a central laboratory and comparative measurements were obtained on the predicate device (ARCHITECT STAT Myoglobin Immunoassay on the ARCHITECT I System). For lithium plasma, 165 samples (including nine spiked samples) were evaluated and the total range of

samples tested was 22 to 882 ng/ml. For whole blood, 157 samples (no spiked samples), with a sample range of 24 to 856 ng/ml, were evaluated. The data was evaluated by the Passing-Bablok linear regression analysis and the results were as follows.

Plasma samples: $y = 1.02x + 13$, $r^2 = 0.99$, n = 165
Whole blood samples: $y = 1.07x + 15$, $r^2 = 0.99$, n = 157

b. Matrix comparison:

In order to demonstrate equivalence of myoglobin results between different anticoagulant combinations, sixty four individual and five spiked samples spanning the measuring range (24 to 889 ng/mL) were evaluated. Four matrix anticoagulant combinations were evaluated in the study, which were Lithium-Heparin whole blood, Lithium-Heparin plasma, EDTA whole blood and EDTA plasma. The results of plasma versus whole blood were analyzed by Passing-Bablock regression. Results are summarized below:

Li-Hep Plasma vs. Li-Hep WB: $y = 0.99x – 1.0$, $r^2 = 1.00$, n = 69
EDTA Plasma vs. EDTA WB: $y = 1.00x – 0.5$, $r^2 = 1.00$, n = 69
EDTA WB vs. Li-Hep Plasma: $y = 1.02x – 0.9$, $r^2 = 1.00$, n = 64
EDTA Plasma vs. Li-Hep Plasma: $y = 1.01x + 0.3$, $r^2 = 1.00$, n = 64

Abbreviations Used: Lithium-Heparin = Li-Hep and Whole Blood = WB

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:
The 97.5th Percentile was determined for women (75 ng/mL) and men (142 ng/mL).

5. Expected values/Reference range:
Whole blood (lithium-heparin and EDTA) and plasma (lithium-heparin and EDTA) were obtained from 343 apparently healthy individuals (ages: 21 to 86 years) from a geographically diverse U.S. adult population (182 women and 161 men) and analyzed using the AQT90 FLEX Myo assay. The data is summarized in the following table.

Category	95% Reference Interval (ng/mL)	97.5th Percentile (ng/mL)	90% Confidence Interval for 97.5th Percentile (ng/mL)
Women	20 to 75	75	69 to 82

Men	24 to 142	142	124 to 164

Each laboratory should establish its own reference range.

N. Instrument Name:
AQT90 FLEX analyzer

O. System Descriptions:

1. Modes of Operation:
   A completely closed, fully automated system, using cartridge-based immunoassays in disposable cups. Offers possibility of running 15 different parameters at a time and has a total capacity of 200 tests without replenishment.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X____ or No _____

3. Specimen Identification:
   Samples are identified from the barcode on blood collection test tube. The code is read automatically by the analyzer.

4. Specimen Sampling and Handling:
   Blood is drawn into normal vacuum test tube. The blood collection tube is placed directly in the analyzer. A volume of sample is automatically drawn from the tube through its rubber seal. The still closed tube is discarded. The used immunoassay cup is discarded into a closed waste bin in the analyzer.

5. Calibration:
   The analyzer can be automatically calibrated by means of calibration cartridges at the time of test cartridge lot change.

6. Quality Control:
   AQT FLEX LQC Multi-CHECK, Levels 1, 2 and 3.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

The sponsor conducted testing on plasma and whole blood samples to estimate the carry-over of myoglobin between samples analyzed on the AQT90 FLEX Analyzer. The detected maximum carry-over was 203 ppm for plasma and 253 ppm for whole blood.

Q. Proposed Labeling:

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

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.