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

A. 510(k) Number:

k131307

B. Purpose for Submission:

New Device

C. Measurand:

Fructosamine

D. Type of Test:

Quantitative colorimetric assay

E. Applicant:

Siemens Healthcare Diagnostics Inc.

F. Proprietary and Established Names:

ADVIA® Chemistry Fructosamine (FRUC) Assay

ADVIA® Chemistry Fructosamine Calibrator

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.7470; Glycosylated hemoglobin assay

   21 CFR 862.1150; Calibrator

2. Classification:

   Class II

3. Product code:

   LCP--Assay, glycosylated hemoglobin

JIT—Calibrator, secondary

4. Panel:

   Hematology (81); LCP

   Clinical Chemistry (75); JIT

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The ADVIA® Chemistry Fructosamine Assay

   For *in vitro* diagnostic use in the quantitative measurement of glycated protein (fructosamine) in human serum or plasma on the ADVIA® Chemistry systems. Measurement of fructosamine is representative of blood glucose levels over the preceding 2-3 weeks and is useful for monitoring diabetic patients.

   ADVIA® Chemistry Fructosamine Calibrator

   For *in vitro* diagnostic use in the calibration of the ADVIA® Chemistry Fructosamine (FRUC) assay on ADVIA® Chemistry systems.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Siemens ADVIA 1650 Chemistry System

I. Device Description:

The ADVIA® Chemistry Fructosamine reagents are ready-to-use liquid packaged for use on ADVIA® 1650 Chemistry system. The reagents are supplied as 100 tests/wedge, with 2 wedges in each kit.

Reagent 1 (R1) contains Tris Buffer (0.2 mol/L, pH 8.0), Proteinase-K (≥ 1 kU/mL) 4-Aminoantipyrine (5 mmol/L) and Stabilizers.

Reagent 2 (R2) contains Tris Buffer (0.2 mol/L, pH 8.65), Fructosaminase (≥ 0.5 kU/mL), Peroxidase (horseradish) (0.5 kU/mL) N-ethyl-N-sulphohydroxypropyl-m-toluidine (TOOS) (10 mmol/L) and Stabilizers.

The ADVIA® Chemistry Fructosamine Calibrator is a single analyte and single level calibrator. It is lyophilized human serum containing pure fructosamine antigen. There are 3 vials (containing 0.08g) in each kit. The volume per vial (after reconstitution with deionized water) is 1.0 mL.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Diazyme Glycated Serum Protein Assay

   Randox Fructosamine Calibrator

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

   k042193 (Assay)
   k023763 (Calibrator)

3. Comparison with predicate:

Similarities and Differences: Assay		
Item	**Candidate Device ADVIA Chemistry Fructosamine Assay**	**Predicate Device Diazyme Glycated Serum Protein Assay (k042193)**
Intended Use	Same	For *in vitro* diagnostic use in the quantitative measurement of glycated protein (fructosamine) in human serum or plasma on the ADVIA® Chemistry systems.
Measurement	Same	Quantitative
Specimen Types	Human Serum and plasma (Lithium heparin, potassium EDTA	Serum
Assay Principle/Methodology	Same	Enzymatic Reaction
Instrument Used	ADVIA 1650 Chemistry System	Chemistry System
Format	Liquid	Lyophilized
Analytical Range	30-1000 µmol/L	21-1354 µmol/L

Similarities and Differences: Calibrator		
Item	**Candidate Device ADVIA Chemistry Fructosamine Calibrators**	**Predicate Device Randox Fructosamine Assay (k023763)**
Intended Use	Same	Intended for *in vitro* use in the calibration of Fructosamine on clinical chemistry systems.
Form	Same	Lyophilized
Analyte Source	Same	Derived from human source
Target Levels	400 µmol/L	521 µmol/L
Fill Volume	Same	0.08g to 1 mL reconstituted
Storage	Same	2-8°C

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

CLSI EP7-A2: Interference Testing in Clinical Chemistry

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods

L. Test Principle:

In the ADVIA® Chemistry Fructosamine (FRUC) assay, Reagent 1 contains proteinase K, which digests the glycated protein to yield glycated protein fragments. Fructosaminase in Reagent 2 oxidizes the ketoamine bond of the glycated protein fragments. As a result, hydrogen peroxide is released and it is involved in a colorimetric Trinder end-point reaction. The amount of color developed and measured at 596 nm is proportional to the concentration of glycated protein in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:
   Precision studies were performed in accordance with the CLSI EP5-A2 guideline. Within run and total imprecision were assessed by testing a serum-based commercially available 2-level control with approximate fructosamine concentrations of 150 and 400 µmol/L. Five additional serum pools spiked with fructosamine antigen containing concentrations ranging from 60-736 µmol/L were also tested, where each

sample was tested in replicates of two, twice daily for 20 days (n=80). Two ADVIA 1650 analyzers and 2 lots of ADVIA fructosamine reagents and calibrators were used in the study. Results of both reagent lots are shown below:

Sample	# Days	# Runs	# Reps	Mean (µmol/L)	Within Run		Between		Between Day		Total	
					SD	%CV	SD	%CV	SD	%CV	SD	% CV
CONTROL 1	20	800	160	150	0.5	0.4	1.1	0.7	1.9	1.2	2.2	1.5
CONTROL 2	20	800	160	428	1.2	0.3	1.9	0.4	345	0.8	4.2	1.0
Serum Pool 1	20	80	160	272	0.9	0.3	1.9	0.7	1.5	0.6	2.7	1.0
Serum Pool 2	20	80	160	729	2.0	0.3	2.1	0.3	4.9	0.7	5.9	0.8
Serum Pool 3	20	80	160	69	0.5	0.7	1.1	1.6	1.5	2.2	1.9	2.8
Serum Pool 4	20	80	160	126	0.6	0.5	1.7	1.3	1.4	1.1	2.3	1.8
Serum Pool 5	20	80	160	539	1.1	0.2	3.6	0.7	3.1	0.6	5.0	0.9
Serum Pool 6	20	80	160	1377	3.5	0.3	9.6	0.7	6.4	0.5	12.3	0.9
Serum Pool 7	20	80	160	1691	3.0	0.2	7.4	0.4	9.6	0.6	13.3	0.9

Additionally, imprecision at the low end of the assay was estimated from a separate study, using 2 low fructosamine pools (30-50 µmol/L) tested twice daily with 2 reagent lots, 2 ADVIA 1650 analyzers and 4 replicates/run over 5 days (total 40-replicates/sample). Results of both reagent lots are shown below:

Sample	# Days	# Runs	# Reps	Mean (µmol/L)	Within Run		Between		Between Day		Total	
					SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Sample 1	5	400	160	39	2.5	2.5	0.6	1.6	0.0	0.0	1.4	3.5
Low Sample 2	5	400	160	47	3.1	6.6	2.4	5.0	0.0	0.0	4.3	9.2

b. Linearity/assay reportable range:

A linearity study was performed using 9 diluted samples with fructosamine concentrations evenly distributed throughout the assay range using 2 lots of reagent and one lot of calibrator. Samples were prepared from a high serum pool spiked with human fructosamine (~1070 µmol/L**)** and low analyte serum pools (fructosamine concentration below 30 µmol/L) to cover the assay range by dilution. The range of samples tested was 27-1070 µmol/L. The observed values versus the expected values range from 100.0-107.4% for both reagent lots.

The linear regression equation is as follows:
$y=0.99x+4.1$, $r= 1.0$

The study supports that the fructosamine assay is linear across the range of 30 to 1000 µmol/L.

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

Traceability:

The ADVIA® Chemistry Fructosamine assay is traceable to an internal standard manufactured using highly purified fructosamine, referred to as the master lot. The master lot was prepared by spiking the appropriate amounts of stock solution containing pure fructosamine antigen into a human serum matrix. The master lot was subsequently value assigned as noted below.

Value Assignment:

The initial lot of calibrator materials (master lot) was value assigned through a correlation study using control and patient samples with values determined using the ADVIA fructosamine assay on the ADVIA 1650 system and Randox fructosamine assay on the Hitachi 717. Based on the correlation study with this method, the fructosamine values for the Master Lot were established. All subsequent lots are value assigned against the Master Lot on the ADVIA 1650 system.

Stability:

Protocols and acceptance criteria were reviewed for the shelf life and closed vial stability of the ADVIA Fructosamine Chemistry Calibrator and found to be acceptable. Reagent: On-board reagent stability is 60 days. The shelf life of the ADVIA Chemistry Fructosamine Reagent is 12 months at 2-8°C. For unopened product, see the expiration date on the reagent carton. Calibrator: for opened products, once the cap is removed, assigned values are stable for 28 days when recapped immediately after use and stored at 2-8°C. The shelf life of the ADVIA Chemistry Fructosamine Calibrator is 12 months at 2-8°C. For unopened product, see the expiration date on the calibrator carton.

d. *Detection limit:*

The Limit of Blank (LoB) and Limit of Detection (LoD) were determined by assaying a serum-like pool and low fructosamine samples according to CLSI EP17-A. The serum-like pool was prepared in 10 mM phosphate buffer (pH 7.4) containing NaCl, 100 mg/dL glucose, 6% human albumin and 1% human immunoglobulin. The low fructosamine LoB pool was spiked with known concentrations of analyte ranging from 18 to 46 µmol/L. LoB and LoD determinations were based on 4 replicate measurements assessed twice daily, using 2 reagent lots over 5 days for a total of 40 replicates/reagent lot (N=80) on the ADVIA 1650 instrument. The observed LoB and LoD values are 15 and 21, respectively.

e. *Analytical specificity:*

Interference in the fructosamine assay was evaluated using 3 human serum pools with

fructosamine concentrations of approximately 150, 250 and 1000 µmol/L and spiked with various interferents. The levels for testing were prepared by diluting the pool with the highest concentration of interferent with the same pool without interferent (control). Five equally spaced dilutions were tested for each interferent and assayed in duplicate using one reagent lot on one ADVIA 1650 system. The following interferents were tested: Bilirubin (unconjugated and conjugated), hemoglobin, lipemia (from triglyceride concentrate), abscorbic acid, glucose and uric acid. The effect of albumin and total blood protein was evaluated by spiking fructosamine into unaltered human patient serum samples with varying levels of albumin (2.8-6.1 g/dL) or total protein (3.2-8.4 g/dL). The assay was considered to have no significant interference if the bias between control and observed fructosamine concentration containing interferent does not exceed 10%. The level at which there is no significant interference for each analyte is listed below:

Interferent	Concentration at Which No Significant Interference Observed (mg/dL):
Bilirubin (conjugated)	5
Bilirubin (unconjugated)	5
Hemoglobin	250
Lipemia	1000
Abscorbic Acid	10
Glucose	2800
Uric Acid	50
Albumin	6100
Total Protein	8400

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:
A total of 113 human serum samples were obtained for the method comparison study. One hundred and ten samples in total were tested, with 10 samples diluted with a zero analyte serum pool to achieve concentrations throughout the assay range. However, 3 samples were not tested since fructosamine concentrations exceeded the analytical measuring range, leaving 110 samples for analysis. All 110 samples were tested in duplicate using the ADVIA fructosamine assay on the ADVIA 1650 Chemistry system and the Diazyme Glycated Serum Protein Enzymatic Assay Kit on the Olympus AU640. The linear regression analysis using the first set of replicates is summarized below:

Regression type	N	Equation	R	Sample range (x) (µmol/L)
Least Squares Linear Regression (First Replicate)	110	y = 0.99x -13.1	0.99	47-995

b. Matrix comparison:

A total of 152 lithium heparin plasma samples and 128 potassium EDTA plasma samples were used for the matrix comparison study with serum samples. Sixteen samples were altered for both sample types to achieve concentrations throughout the measuring range. All samples were tested in duplicate by one operator using the ADVIA Fructosamine assay on the ADVIA 1650 Chemistry system. The linear regression analysis using the first set of replicates is summarized below:

Sample Type	Regression type	N	Equation	R	Sample range (x) (µmol/L)
Plasma (Lithium Heparin)	Least Squares Linear Regression (First Replicate)	152	y = 1.00x + 3.5	0.99	38 - 940
Plasma (Potassium EDTA)	Least Squares Linear Regression (First Replicate)	128	y = 1.00x – 4.6	0.99	37 - 940

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 expected normal range for non-diabetic adult fructosamine is 122-236 µmol/L according to the literature cited by the Sponsor*. This reference range was confirmed using the ADVIA Chemistry Fructosamine assay on the ADVIA 1650 Chemistry system by assaying 25 serum samples collected in-house from healthy adults. The samples were assayed in duplicate using one reagent lot on 1 ADVIA system by 1 operator.

   *O'Brien JE, Brookes M. *Determination of Reference Values for a Novel Ketoamine-Specific Fructosamine Assay for Assessment of Diabetic Glycemic Control*. Diabetes Technology and Therapeutics, 1999; 1:447-455.

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.