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

A. 510(k) Number:

k081895

B. Purpose for Submission:

New device

C. Measurand:

Glycosylated Hemoglobin (Hgb A1C)

D. Type of Test:

Quantitative, latex agglutination inhibition method

E. Applicant:

Siemens Healthcare Diagnostics Inc.

F. Proprietary and Established Names:

ADVIA Chemistry Hemoglobin A1c Assay

ADVIA Chemistry A1c Calibrators

G. Regulatory Information:

1. Regulation section:

   21CFR 864.7470

   21CFR 864.1150

2. Classification:

   Class II

3. Product code:

   LCP, JIX

4. Panel:

   Hematology (81), Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for use below.

2. Indication(s) for use:

   The ADVIA Chemistry Hemoglobin A1c method is for *in vitro* diagnostic use in the quantitative determination of Hemoglobin A1c, a diabetes marker, in whole blood on the ADVIA Chemistry systems. Such measurements are used for monitoring the long-term glycemic control of persons with diabetes. The A1c and total hemoglobin (tHb) values generated as part of the HbA1cN and HbA1cI results are intended for use in the calculation of the A1c/total hemoglobin ratio, and must not be used individually for diagnostic purposes.

   *Note: HbA1cN reports HbA1c in % and HbA1cI reports HbA1c in mmol/mol

   The ADVIA Chemistry A1c Calibrators are for *in vitro* diagnostic use in the calibration of the A1c and total hemoglobin methods (Automated and Manual Pretreatment) on the ADVIA Chemistry System.

3. Special conditions for use statement(s):

   For prescription use only

   The sponsor has the following limitation stated in their labeling:
   "Fetal Hemoglobin (HbF) consists of two alpha and two gamma chains that are not recognized by the anti-HbA1c antibody. Samples containing high amounts of Hb F (>10%), usually found in some people with Thalassemia, in infants and in some pregnant women, may yield a lower than expected HbA1c result with this method. For blood samples containing HbF (>10%), HbA1c results obtained by this method should not be compared to published normal or abnormal results."

4. Special instrument requirements:

   Siemens ADVIA 1200, 1650, 1800 and 2400 analyzers

I. Device Description:

Reagents

For Automated method, the ADVIA Chemistry A1c Reagent kit consists of four reagents:

1. Reagent 1– contains 2µg/mL of A1c hapten covalently attached polymer, 1.5% bovine serum albumin, buffer, preservative, and sodium azide.
2. Reagent 2 – contains 1.8 mg/L of Anti-hemoglobin A1c coupled latex (mouse), 1.5% bovine serum albumin, buffer and preservative,
3. A1c Denaturant Reagent – contains 0.2% porcine pepsin, buffer and preservative.
4. Total Hemoglobin Reagent – contains 0.4% sodium hydroxide

For Manual Sample Pretreatment method, the ADVIA Chemistry A1c Reagent kit consists of the following:

1. Reagent 1– contains 2µg/mL of A1c hapten covalently attached polymer, 1.5% bovine serum albumin, buffer, preservative, and sodium azide.
2. Reagent 2 – contains 1.8 mg/L of Anti-hemoglobin A1c coupled latex (mouse), 1.5% bovine serum albumin, buffer and preservative,
3. Total Hemoglobin Reagent – contains 0.4% sodium hydroxide
4. Hemoglobin Denaturant Reagent – contains 0.01% porcine pepsin, buffer and preservative.

Calibrators:

The ADVIA® Chemistry A1c Calibrators are used to calibrate the methods. The calibrators consist of four (4) levels of lyophilized whole blood containing varying concentrations of HbA1c and total hemoglobin. There is a single level calibration for total hemoglobin (Cal 1) and a multi-level calibration (six levels) for A1c. Four calibrator levels (designated Cal 1 – 4) are in a single kit. The other two levels consist of Saline (Cal 0) and Cal 5 (prepared by the system from Cal 4 using 1.4 times the volume used for Cal 4).

The human blood used in the manufacture of these calibrators has been tested using FDA approved methods and found to be non-reactive for HBsAg and antibodies to HCV and HIV-1/2.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Tosoh G7 Automated HPLC analyzer-HbA1c Variant Analysis Mode

   Ortho-Clinical Diagnostic VITROS Chemistry Products Calibrator Kit 18

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

   k011434, k041764

3. Comparison with predicate:

ADVIA Chemistry Hemoglobin A1c Assay

Similarities and Differences:

	ADVIA Chemistry Hemoglobin A1c ***(candidate device)***	**Tosoh G7 Automated HPLC Analyzer – HbA1c Variant Analysis Mode** ***(predicate device)***
Intended Use	For *in vitro* diagnostic use for the measurement of hemoglobin A1c	For *in vitro* diagnostic use for the measurement of hemoglobin A1c
Specimen Type	Human Whole Blood (EDTA and Lithium Heparin)	Human Whole Blood
Standardization	Traceable to NGSP, Traceable to IFCC by Master Equation	Traceable to NGSP
Assay Principle	For Hemoglobin: Conversion of all hemoglobin derivatives into alkaline hematin For HbA1c: Latex agglutination inhibition	High Performance Liquid Chromatography (non-porous ion exchange)
Results determination	Ratio of HbA1c/ Total Hemoglobin	Measurement of HbA1c as a percentage of total hemoglobin
Calibration	Multipoint calibration (6 levels) for Hemoglobin A1c, single point calibration for Total Hemoglobin	2 point Calibration (high and low)
Analytical Range	2.9 – 15.4%	4.2 – 20.8%

ADVIA Chemistry A1c Calibrators

Similarities and Differences:

	ADVIA Chemistry A1c Calibrators ***(candidate device)***	**VITROS Chemistry Products Calibrator Kit 18** ***(predicate device)***
Intended Use	For *in vitro* diagnostic use in the calibration of the A1c and total hemoglobin methods (Automated and Manual Pretreatment) on the ADVIA Chemistry Systems	For *in vitro* diagnostic use only. VITROS Chemistry Products Calibrator Kit 18 is used in conjunction with VITROS Chemistry Products FS Calibrator 1 to calibrate VITROS 5, 1 FS Chemistry Systems for the calculation of percent glycated hemoglobin (%A1c).

Calibrator Levels	4 levels provided	4 levels provided
Matrix / Ingredients	Processed human whole blood	Hemolysate derived from human and ovine blood, surfactants, stabilizer, and preservatives
Form	Lyophilized	Lyophilized
Storage	2 – 8°C	2 – 8°C
Reconstituted Vial / Use Stability	7 days stored at 2 – 8°C	2 days stored at 2 – 8°C
Preparation	Reconstitute with DI water before use	Reconstitute with diluent before use

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

None were referenced.

L. Test Principle:

The ADVIA Chemistry Hemoglobin A1c assay measures the concentration of A1c and total hemoglobin and reports the ratio in either % or mmol/mol. There are two different sample pretreatment methods available on the ADVIA Chemistry system.

1.) The first method is an automated sample pretreatment that uses 4 reagents: A1c Denaturant Reagent, A1c Agglutinator Reagent (R1), A1c Antibody Reagent (R2), and Total hemoglobin Reagent (tHb-2). In this automated pretreatment step, the whole blood sample is mixed with the A1c Denaturant Reagent. The red blood cells are lysed and the hemoglobin chains are hydrolyzed by the protease present in the reagent. The HbA1c present in the sample then competes with the agglutinator for the anti-HbA1c antibody; thereby reducing the rate of agglutination. A concentration curve is obtained by monitoring the change in scattered light as a change of absorbance. The actual change in absorbance is inversely proportional to the concentration of HbA1c in the sample.

For the measurement of total hemoglobin, the Total Hemoglobin Reagent (tHb-2) is used. The method is based on the conversion of all hemoglobin derivatives into alkaline hematin in an alkaline solution of a nonionic detergent as described in the original procedure of Wolf, Lang, and Zander.

ii.) The second method is a manual sample pretreatment that uses the same reagents as the first method except that the A1c Denaturant Reagent is replaced with the Hemoglobin Denaturant Reagent. For this method, there is an off-line pretreatment that is followed by ten minute incubation.

Two ratio calculations are provided to the customer. The first (HbA1cN) reports results in NGSP equivalent units (%) while the second ratio (HbA1cI) reports results in IFCC equivalents units (mmol/mol).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A precision study was performed by testing two whole blood samples (a low and a high level) and two levels of control materials. Each sample was assayed on the ADVIA 1200, 1650/1800 and 2400 analyzers for both methods (Automated and Manual Pretreatment) in duplicate per run with 2 runs per day for 20 days (N=80). The results are shown below:

ADVIA 1200 (Automated Pretreatment)

Sample	N	Mean (%A1c)	Within Run		Total imprecision	
			SD	%CV	SD	%CV
Control 1	80	5.49	0.15	2.8	0.19	3.4
Control 2	80	9.57	0.26	2.7	0.27	2.8
Normal blood pool	80	5.46	0.17	3.1	0.24	4.4
High blood pool	78	12.99	0.18	1.4	0.25	2.0

ADVIA 1200 (Manual Pretreatment)

Sample	N	Mean (%A1c)	Within Run		Total imprecision	
			SD	%CV	SD	%CV
Control 1	80	5.08	0.17	3.3	0.22	4.3
Control 2	80	9.14	0.17	1.8	0.22	2.4
Normal blood pool	80	5.18	0.17	3.2	0.20	3.8
High blood pool	80	12.54	0.14	1.1	0.19	1.5

ADVIA 1650/1800 (Automated Pretreatment)

Sample	N	Mean (%A1c)	Within Run		Total imprecision	
			SD	%CV	SD	%CV
Control 1	80	5.54	0.08	1.5	0.11	1.9
Control 2	80	9.45	0.08	0.8	0.12	1.3
Normal blood pool	80	5.41	0.10	1.9	0.19	3.5
High blood pool	80	12.84	0.10	0.8	0.16	1.3

ADVIA 1650/1800 (Manual Pretreatment)

Sample	N	Mean (%A1c)	Within Run		Total imprecision	
			SD	%CV	SD	%CV
Control 1	80	5.05	0.05	1.0	0.10	1.9
Control 2	80	8.85	0.05	0.6	0.12	1.4
Normal blood pool	80	5.05	0.04	0.8	0.08	1.6
High blood pool	80	12.58	0.08	0.7	0.14	1.1

ADVIA 2400 (Automated Pretreatment)

Sample	N	Mean (%A1c)	Within Run		Total imprecision	
			SD	%CV	SD	%CV
Control 1	80	5.28	0.09	1.7	0.18	3.3
Control 2	80	8.99	0.16	1.7	0.27	3.0
Normal blood pool	80	5.14	0.12	2.4	0.17	3.3
High blood pool	80	12.49	0.31	2.5	0.35	2.8

ADVIA 2400 (Manual Pretreatment)

Sample	N	Mean (%A1c)	Within Run		Total imprecision	
			SD	%CV	SD	%CV
Control 1	80	5.08	0.05	0.9	0.12	2.4
Control 2	80	8.81	0.06	0.6	0.18	2.0
Normal blood pool	80	5.05	0.03	0.6	0.12	2.4
High blood pool	80	12.42	0.12	0.9	0.21	1.7

b. Linearity/assay reportable range:

A linearity study across the entire measuring range was assessed using commercially available linearity set solutions. The low and high levels of the linearity set were mixed to make seven intermediate levels and all samples were tested on the various platforms using the ADVIA Chemistry HbA1c. The range of samples tested was from 2.9-15.4% A1c. The observed values were compared to the expected values. The percent recovery between the observed values verses the expected values for all the platforms are shown in the table below. In addition, linear regressions correlations summary for all platforms (observed vs. expected values) are shown in the table below:

Percent recovery table:

Platforms/Methods	Percent recovery ranges for all observed values vs. the expected values:
ADVIA 1200 Automated	93.5-102.5%
ADVIA 1200 Manual	96.3-104.3%
ADVIA 1650/1800 Automated	90.3-104.9%
ADVIA 1650/1800 Manual	96.6-101.8%
ADVIA 2400 Automated	90.3-106.6%
ADVIA 2400 Manual	96.1-104.8%

Linear regression table:

Platforms/Methods	Regression equation	R
ADVIA 1200 Automated	y = 1.09x - 0.27	0.998
ADVIA 1200 Manual	y = 1.06x – 0.30	0.999
ADVIA 1650/1800 Automated	y = 1.09x - 0.45	0.999
ADVIA 1650/1800 Manual	y = 1.05x - 0.23	0.999
ADVIA 2400 Automated	y =1.08x - 0.35	0.998
ADVIA 2400 Manual	y = 1.09x - 0.9	0.998

The data provided support the sponsor's claims that the reportable range of this assay is 2.9-15.4% A1c.

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

Traceability:

The assigned A1c and total hemoglobin (tHb) values of the calibrators are traceable to the NGSP reference method (Tosoh G7) through a correlation study. The ADVIA Chemistry Hemoglobin A1c assay is certified with the National Glycohemoglobin Standardization Program (NGSP). The NGSP certification expires in one year. See NGSP website for current certification at http://www.ngsp.org.

The derived result of the ratio (%) from the NGSP correlation is calculated from the individual quantitative results for total hemoglobin (tHb) and Hemoglobin A1c (HbA1c). The International Federation of Clinical Chemistry (IFCC) units of mmol/mol are calculated using the Master Equation:

IFCC = (NGSP- 2.15) / 0.092

Two different units are provided to the customers:

NGSP equivalent units (%) and IFCC equivalents units (mmol/mol)

Value assignment:

The initial lot of calibrators (master lot) was value assigned through a correlation study using samples provided by the NGSP with values determined on the Tosoh G7. Based on the correlation study with the NGSP Reference method, the total hemoglobin and A1c values for the Master Lot were established. All subsequent lots will be value assigned against the Master Lot.

Stability:

The shelf life and open vial stability testing protocols for the calibrators and the acceptance criteria were described and found to be acceptable. Calibrators are stable until expiration date when stored at 2-8^{o}C and stable for 7 days at 2-8^{o}C once reconstituted with deionized water.

d. Detection limit:

The Limit of Blank (LoB) and Limit of Detection (LoD) were determined by assaying a zero sample and a low sample according to the CLSI guideline EP17-A. Each sample was assayed twice a day in duplicate for 20 days (N=80) on the ADVIA systems. The detection limits were summarized in the table shown below.

Platforms/Methods	**LoB (% A1c)**	**LoD (%A1c)**
ADVIA 1200 Automated	1.2	1.7
ADVIA 1200 Manual	1.0	1.5
ADVIA 1650/1800 Automated	0.6	0.7
ADVIA 1650/1800 Manual	0.5	0.7
ADVIA 2400 Automated	0.6	0.9
ADVIA 2400 Manual	0.9	1.1

This assay has a reportable range of 2.9% to 15.4% A1c.

e. Analytical specificity:

i.) Studies were performed to assess common or known substances that could interfere with the ADVIA A1c assay on the ADVIA 1650/1800 analyzer using the Automated Pretreatment and Manual Pretreatment methods. The interfering substances were evaluated in whole blood EDTA samples that had different Hgb A1C concentrations (6% and 9%). The sponsor's acceptable criterion is that the

analyte recovery should not vary from the base recovery by no more than 10%. No significant interference was defined as the % recovery of ≤ 10%.

The sponsor claimed that there was no significant interference by the following interferents:

- Conjugated and unconjugated bilirubin up to 60.0 mg/dL
- Triglycerides (Intralipid) up to 500 mg/dL
- Rheumatoid factor up to 1100 IU/mL

ii.) An interference study was performed to assess the effect of labile A1c with the ADVIA A1c assay. Two levels of pooled whole blood samples (~5% and 10% A1c) were used and each pool was split into two aliquots. One aliquot was used as the control sample while the other aliquot was supplemented by dissolving 0.030 g (30mg) of glucose into the pool sample (glucose concentration = 1500 mg/dL). The sample was incubated for five hours at 37°C to facilitate formation of labile A1c. Both aliquots were tested on the ADVIA 1800 analyzer using the Automated Pretreatment and Manual Pretreatment methods. The sponsor's acceptance criterion is ≤ 10% bias between the tested and the control samples.

The sponsor concluded that labile A1c concentrations do not interfere with the ADVIA A1c assay.

iii.) An interference study was performed to assess the effect of carbamylated A1c with the ADVIA A1c assay. Two levels of pooled whole blood samples (~5% and 10% A1c) were used and each pool was split into two aliquots. One aliquot was used as the control sample while the other aliquot was supplemented by dissolving 3 mg of urea into the pool sample (urea concentration = 150 mg/dL). The sample was incubated for two hours at 37°C to facilitate formation of carbamylated A1c. Both aliquots were tested on the ADVIA 1800 analyzer using the Automated Pretreatment and Manual Pretreatment methods. The sponsor's acceptance criterion is ≤ 10% bias between the tested and the control samples.

The sponsor concluded that carbamylated A1c concentrations do not interfere with the ADVIA A1c assay.

iv.) A hemoglobin concentration interference study was done on the ADVIA 1800 by testing varying concentrations of total hemoglobin at the same level of %A1c. Five individual normal donors were used for the study. The acceptance criterion for the hemoglobin interference was %HbA1c at different hemoglobin levels must be within 0.95-1.05% of target %HbA1c. The results met the sponsor's predetermined acceptance criteria and the sponsor concluded that total hemoglobin in the range of 7 to 26 g/dL does not interfere with the ADVIA A1c assay.

v.) A hemoglobin variant interference study was carried out using NGSP samples known to be Hemoglobin variants C, E, F and S. These variant samples were

tested on the Primus Ultra 2 HPLC system and the ADVIA 1200, 1650/1800, and 2400 using the automated pretreatment method. All the variants tested showed <12% bias except Hemoglobin F at concentrations of F greater than 10%. Hemoglobin F variant >10% showed a negative bias therefore, the sponsor has the following limitation stated in their labeling:

"Fetal Hemoglobin (HbF) consists of two alpha and two gamma chains that are not recognized by the anti-HbA1c antibody. Samples containing high amounts of Hb F (>10%), usually found in some people with Thalassemia, in infants and in some pregnant women, may yield a lower than expected HbA1c result with this method. For blood samples containing HbF (>10%), HbA1c results obtained by this method should not be compared to published normal or abnormal results."

See NGSP at http://www.ngsp.org for assay interferences with hemoglobin variants.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed in conjunction with the NGSP guidelines for manufacturer certification using the reference laboratory. The study was completed using 80 (two sets of forty frozen whole blood samples in EDTA) patients split between the reference lab and the sponsor. Each sample was analyzed in duplicate on each ADVIA Chemistry System (ADVIA 1200, 1650/1800 and 2400) using both sample pretreatment formats (Automated and Manual Pretreatment methods). After completion of the testing, the reference lab provided the sponsor with the sample values obtained on the Tosoh G7 HbA1c HPLC system (predicate device). Sample range tested was 4.4%- 12.6% A1c. The linear regression correlation using only the first set of replicates (singlicate values) are summarized as follows:

Platforms/Methods	Regression Equations	R
ADVIA 1200 Automated	y= 0.99x + 0.16	0.991
ADVIA 1200 Manual	y= 0.98x + 0.24	0.992
ADVIA 1650/1800 Automated	y= 0.98x + 0.20	0.996
ADVIA 1650/1800 Manual	y= 0.95x + 0.27	0.995
ADVIA 2400 Automated	y= 0.95x + 0.16	0.996
ADVIA 2400 Manual	y=0.94x + 0.35	0.995

In addition, a separate comparison study was performed between the candidate device and another commerically available device using the automated method on the ADVIA 2400 analyzer. The linear regression correlation is summarized in the table below:

Platforms/Methods	N	Regression Equations	R	Samples range tested
Variant II Turbo	139	y= 1.03x – 0.30	0.997	4.1-14.2 %

b. Matrix comparison:

A total of 40 random matched sample pairs (Potassium EDTA, Lithium heparin) were tested on the ADVIA 1650/1800 using the Automated and Manual Pretreatment methods. The linear regression correlation was calculated as follows:

For Automated pretreatment method:

y= 0.98x + 0.07, r= 0.991, sample range tested = 4.7-7.9%

For Manual pretreatment method:

y= 0.97x - 0.02, r= 0.998, sample range tested = 4.8-12.1%

(x=Potassium EDTA, y=Lithium heparin)

The sponsor recommends that EDTA and Lithium heparin can be used as anticoagulants.

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 %HbA1c value is 4.0-6.0 % (20-92 mmol/mol)* according to the literature that the sponsor cited.

*Wu AHB. *Tietz Clinical Guide to Laboratory Tests, 4th edition,* Saunders Elsevier, St. Louis, MO 480-483 (20006).

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.