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

A. 510(k) Number:

k063306

B. Purpose for Submission:

New device

C. Measurand:

Hemoglobin A1c

D. Type of Test:

Quantitative assay

E. Applicant:

Alfa Wassermann Diagnostic Technologies, LLC

F. Proprietary and Established Names:

ACE® Hemoglobin A1c (HbA1c) Reagent
Hemoglobin A1c Calibrators
Hemoglobin A1c Controls

G. Regulatory Information:

1. Regulation section:

   864.7470, Glycosylated Hemoglobin Assay
   862.1150, Calibrator, Secondary
   862.1660, Quality Control Material

2. Classification:

   Class II, Class II, and Class I, respectively

3. Product code:
    LCP, JIT, JJX, respectively

4. Panel:

    81 (Hematology), 75 (Chemistry), and 75 (Chemistry), respectively

H. Intended Use:

1. Intended use(s):

    See indications for use below.

2. Indication(s) for use:

    ACE® Hemoglobin A1c (HbA1c) Reagent is intended for the quantitative determination of hemoglobin A1c (µmol/L) and total hemoglobin (g/dL) in human whole blood (EDTA) for the calculation of percent hemoglobin A1c using the ACE clinical chemistry system. This test is intended for use in clinical laboratories or physician office laboratories to monitor long term blood glucose control in individuals with diabetes mellitus. For *in vitro* diagnostic use only.

    The µmol HbA1c and total hemoglobin (THb) values generated are intended for use in the calculation of the HbA1c / THb ratio, and cannot be used individually for diagnostic purposes.

    Hemoglobin A1c Calibrators are intended for use in the performance of both a multi-point calibration for hemoglobin A1c and a single-point calibration of total hemoglobin on the ACE® clinical chemistry system, for the quantitative determination of percent (%) hemoglobin A1c in whole blood. For *in vitro* diagnostic use only.

    Hemoglobin A1c Controls are intended to reliably monitor the accuracy and precision of quantitative determinations of hemoglobin A1c on the ACE® clinical chemistry system. For *in vitro* diagnostic use only.

3. Special conditions for use statement(s):
    The assay is for prescription use and is intended for use in point-of-care settings.

4. Special instrument requirements:

    The device is intended for use on the ACE Analyzer.

I. Device Description:

The ACE Hemoglobin A1c Reagent is provided as a single kit and consists of four bottles containing a hemoglobin denaturant, a total hemoglobin reagent, a HbA1c agglutinator reagent and a HbA1c antibody (mouse monoclonal) reagent.

The ACE Hemoglobin A1c Calibrators are provided as a single kit and contain six ready-to-use liquid calibrators and consist of buffer matrix spiked with known concentrations of hapten. Levels 2-6 range from 0.3 to 8.8 μmol/L of HbA1c. Calibrator 1, for the total hemoglobin portion of the assay, consists of sodium hydroxide and Chlorohemin prepared to a concentration of 19.9 g/dL hemoglobin. The ACE Hemoglobin A1c Controls are lyophilized human blood hemolysate. They are provided as a single kit and contain normal and elevated levels of HbA1c and a reconstitution fluid. Separate package labeling is provided for both the calibrators and controls.

The controls are prepared from human whole blood. The sponsor indicates that the controls have been tested and found negative for antibody to Human Immunodeficiency Virus (anti-HIV) Types 1 and 2, antibody to Hepatitis C (anti-HCV) and for Hepatitis B Surface Antigen (HBsAg) by FDA recommended (approved/licensed) tests.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Tosoh Bioscience, Inc. A1c 2.2 Plus Automated Glycohemoglobin Assay
   General Atomics, Inc. Hemoglobin A1c Enzymatic Assay

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

   k972265 and k050178, respectively

3. Comparison with predicate:
   Both devices are for measurement of the same analyte in the same matrix, have the same intended use, and similar reference ranges. They utilize different test methodologies (i.e., latex agglutination inhibition and HPLC).

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

The sponsor referenced the following guidance document(s) or standards:

Review Criteria for Assessment of Glycohemoglobin (Glycated or Glycosylated) Hemoglobin In Vitro Diagnostic Devices, 1997.

CLSI document EP5-A. Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline.

CLSI document EP7-A. Interference Testing in Clinical Chemistry; Approved Guideline.

CLSI document EP6-A. Evaluation of the Linearity of Quantitative Measurement Procedure: A Statistical Approach; Approved Guideline.

The sponsor did not indicate any deviations from these guidances.

L. Test Principle:

Prior to placing the patient samples on the ACE clinical chemistry analyzer whole blood samples require a pretreatment step. The red blood cells in the sample are lysed by the Hemoglobin Denaturant and the hemoglobin chain hydrolyzed.

For determination of HbA1c, a latex agglutination inhibition assay is used. In the absence of HbA1c in the sample, the agglutinator (synthetic polymer containing the immunoreactive portion of HbA1c) in the HbA1c Agglutinator Reagent and the antibody-coated microparticles in the HbA1c Antibody Reagent will agglutinate. The presence of HbA1c in the sample competes for the antibody binding sites and inhibits agglutination. The increase in absorbance, monitored monochromatically at 592 nm on the ACE clinical chemistry system, is inversely proportional to the HbA1c present in the sample. For the determination of total hemoglobin, all hemoglobin derivatives in the sample are converted to alkaline hematin. The reaction produces a green colored solution, which is measured bichromatically at 573 nm/692 nm. The intensity of color produced is directly proportional to the total hemoglobin concentration in the sample. The ratio between HbA1c and total hemoglobin is then calculated to obtain the percent HbA1c value.

For the determination of total hemoglobin, all hemoglobin derivatives in the sample are converted to alkaline hematin. Total Hemoglobin Reagent is first loaded on the ACE analyzer. After instrument calibration is performed with the Alfa Wassermann Total Hemoglobin Calibrator, controls and patient samples are loaded on the instrument. The instrument then mixes the sample and reagent in a cuvette, and after an incubation period the absorbance change in the cuvette is monitored. The reaction is measured bichromatically at 573 nm/692 nm. The intensity of color produced is directly proportional to the total hemoglobin concentration in the sample. The ACE analyzer calculates the concentration of total hemoglobin in the sample using the total hemoglobin calibration standard curve.

After both the concentration of HbA1c and total hemoglobin are determined using calibration curves, the ACE analyzer calculates the ratio of the two tests and the result is reported as percent HbA1c.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies were done according to CLSI EP5-A Guidelines. Precision was measured on a normal (6.3% A1c) and elevated (12.2% A1c) patient sample using the ACE clinical chemistry system. The within-run CV for the normal patient was 2.9% and for the elevated patient was 3.0%. The total CV for the normal patient was 7.0% and for the elevated patient was 5.9%.

Precision studies were also done in three separate Physician Office Laboratory (POL) sites. These studies were conducted by personnel without formal medical technology education. The studies consisted of running three EDTA whole blood samples (the same three samples were run at each of the three labs) with varying levels of HbA1c in triplicate on five different days.

Lab	Sample	Mean	Within-Run CV%	Total CV%
A	Low	4.8	2.3%	3.3%
B	Low	5.3	5.4%	5.4%
C	Low	5.1	3.3%	3.6%
A	Mid	7.3	1.8%	2.7%
B	Mid	8.3	2.7%	4.1%
C	Mid	7.8	1.8%	2.4%
A	High	10.9	2.8%	3.3%
B	High	12.3	5.1%	5.1%
C	High	11.6	2.2%	2.9%

b. Linearity/assay reportable range:

Linearity of the ACE Hemoglobin A1c assay was evaluated using the CLSI, EP6 Guideline. Five different samples with %HbA1c assigned values of 0.5-16.9% were tested on the ACE clinical chemistry system. In a separate study, six different samples with assigned % HbA1c values ranging from 5.4% to 21.4% were measured. Linear regression analysis confirmed the claim of linearity between 3.0%-18.0% HbA1c. The results supported device specification of 1% HbA1c (in HbA1c units) or 5% (in percent).

The measuring range for total hemoglobin was verified using a whole blood sample. The sample was serially diluted using the packed cells and plasma from the blood sample. Sample results, ranging from 10-21 g/dL fell within 10% of the expected value using 95% confidence intervals.

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

The sponsor's calibrators and controls are specifically identified for use.

The sponsor claims that the calibrators are traceable to a NGSP reference method. Currently, the ACE® Hemoglobin A1c (HbA1c) Reagent used on the ACE analyzer has not been certified by the NGSP.

Multiple analyzers and lots of reagent are used to analyze control materials for the purpose of establishing their published acceptable ranges. Traditional statistical calculations are used, i.e., mean +/- 2 standard deviations.

Users are instructed in the package insert to follow federal, state, and local guidelines concerning QC practices.

Stability
Stability studies are summarized for the calibrators and/controls. The sponsor specifies the method used to analyze the material, environmental storage conditions, frequency of testing, baseline against which measurements are compared, and acceptance criteria for the study. Accelerated studies are used by the sponsor to estimate the expiration date, and on-going real time studies are being performed. Results vary less than 10% from the expected values throughout the expiration date.

d. *Detection limit:*

Not applicable.

e. *Analytical specificity:*

For interference testing, CLSI EP7A Guidelines were used. To evaluate the effects of bilirubin interference, unconjugated bilirubin was added to a whole blood sample in various concentrations ranging from 1.6-50 mg/dL. Recoveries of % HbA1c at the various concentrations ranged from 96.7 to 101.3%. Using a specification of less than a 10% change in recovery of %HbA1c from the control (no bilirubin added), studies demonstrated that bilirubin up to 50 mg/dL did not interfere with the assay.

For the lipemia interference study, six normo-lipemic and lipemic whole blood samples (up to 2808 mg/dL triglycerides) were tested for %HbA1c on the ACE clinical chemistry system. Packed cells from each sample were washed three times with saline and the samples were re-assayed. Using the specification of less than a 10% change in recovery from the washed %HbA1c value, the change in %HbA1c ranged from 0% to 10%. Studies demonstrated that lipemic samples with triglyceride levels up to 2500 mg/dL did not interfere with this assay.

To evaluate the performance of the assay for testing hemoglobin variants, whole blood samples were obtained which contained Hb C, Hb E, Hb F and Hb S. These samples had previously been characterized by reference methods known to be unaffected by variants. The samples were run on the ACE clinical chemistry system for % HbA1c. The percent recovery was greater than 10% difference in all cases, with

the exception of Hb F, where the percent recovery averaged 106% (n = 3, range 91 to 116%). Results of this study support the sponsor's claims, i.e., that Hb C, Hb E and Hb S interfere with this method and that Hb F may interfere with this method by giving higher than expected results.

For the labile HbA1c interference study, two whole blood samples (one with a normal and one with an elevated % HbA1c level) were incubated with zero (control) and increasing levels of glucose at 37°C for four hours. The samples were then run on the ACE clinical chemistry system. For both the normal and elevated samples, % HbA1c results for all glucose concentrations tested were less than 10% lower than the control results compared to the reference method.

  f. *Assay cut-off:*

   Not applicable.

2. Comparison studies:

  a. *Method comparison with predicate device:*
   A correlation study was performed against the Tosoh Bioscience's A1c 2.2 Plus Automated Glycohemoglobin Assay. The study followed the CLSI EP9-T Guidelines. The Tosoh assay is certified by the National Glycohemoglobin Standardization Program. Forty-two residual human samples were evaluated, ranging in concentration from 4.5-12.2% hemoglobin A1c. The following results were observed:

Method Comparison Data Compared to the Predicate Device

Regression Equation	Correlation Coefficient	Standard Error of the Estimate	Confidence Interval Slope	Confidence Interval Intercept
y = 1.052x+0.23	0.975	0.49	1.000 to 1.104	-0.20 to 0.67

   Studies were also performed at 3 POL Sites. These studies were conducted by personnel without formal medical technology education. The studies consisted of running 20 EDTA whole blood samples (the same 20 EDTA whole blood samples were run at each of the three labs) with varying levels of HbA1c (determined using HPLC) in duplicate in four different runs of ten samples each.

POL Method Comparison Data Compared to HPLC

Lab	Regression Equation	Correlation Coefficient	Standard Error of the Estimate	Confidence Interval Slope	Confidence Interval Intercept
A	Y = 0.940x+0.41	0.9762	0.46	0.871 to 1.009	-0.16 to 0.99
B	Y = 1.102x-0.04	0.9818	0.47	1.032 to 1.172	-0.63 to 0.55
C	Y = 1.035x+0.38	0.9844	0.41	0.974 to1.095	-0.89 to 0.13

b. *Matrix comparison:*

Studies were performed with EDTA samples and this sample type is the only anticoagulant recommended for use.

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):*

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The reference range of the candidate device is 4.3 to 6.3%. ADA Reference range is 4.0-6.0%.

The sponsor established their reference range by analyzing samples from 56 healthy adults, and calculating the 2.5[th] and 97.5[th] percentile values (as recommended in the CLSI/NCCLS C28-A2 document: How to Determine Reference Intervals in the Clinical Chemistry Laboratory; Approved Guideline – Second Edition).
The reference range was also confirmed in an earlier study involving 20 healthy adults.

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

The sponsor presented sample stability data to support their claims. On-going real time studies are being conducted, allowing no more than a 10% shift from the initial value. HPLC and frozen low and high value patient samples are used to monitor the values.

O. Proposed Labeling:

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

P. Conclusion:

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