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

I Background Information:

A 510(k) Number

K221326

B Applicant

Nova Biomedical Corporation

C Proprietary and Established Names

Nova Allegro HbA1c Assay, Nova Allegro Analyzer

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LCP	Class II	21 CFR 864.7470 - Glycosylated Hemoglobin Assay	HE - Hematology
JQT	Class I	21 CFR 862.2400 - Densitometer/scanner (integrating, reflectance, TLC, or radiochromatogram) for clinical use	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

This submission is a Dual 510(k) and CLIA Waiver by Application (Dual Submission) for a new device, tracked as K221326 and CW220003.

B Measurand:

Whole blood glycosylated hemoglobin (HbA1c)

C Type of Test:

Quantitative, latex enhanced turbidimetric immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
The Nova Allegro HbA1c Assay is intended for in vitro diagnostic use on the Nova Allegro Analyzer for the quantitative determination of the glycated Hemoglobin (% hemoglobin A1c) in capillary whole blood obtained from the fingertip.

The results from this assay are intended to be used for the monitoring of long-term blood glucose/metabolic control in individuals with diabetes mellitus.

The Nova Allegro Analyzer is intended for in vitro diagnostic use in clinical laboratory and near-patient testing (point-of-care) settings for the quantitative determination of Nova Allegro Assays using Nova Allegro Test Cartridges.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

- This HbA1c test should not be used for analyzing samples from patients with conditions causing shortened red blood cell survival, such as hemolytic diseases, pregnancy and significant acute or chronic blood loss.
- This HbA1c test should not be used in monitoring daily glucose control and should not be used to replace daily home testing of urine and blood glucose levels.
- This HbA1c test is not intended for use in the diagnosis of or screening for diabetes.
- This HbA1c test is not intended for use on neonates.
- This device has significant negative interference with fetal hemoglobin (HbF). HbA1c results are invalid for patients with abnormal amounts of HbF, including those with known Hereditary Persistence of Fetal Hemoglobin.

D Special Instrument Requirements:

The Nova Allegro HbA1c Assay is used with the Nova Allegro Analyzer.

IV Device/System Characteristics:

A Device Description:

The Nova Allegro HbA1c Assay is a fully automated assay for the determination of the percentage of HbA1c in capillary fingerstick whole blood and the calculation of estimated average glucose (eAG). The Allegro HbA1c Test Cartridge contains all reagents required for the determination of % HbA1c and includes an integrated capillary sample collection device.

The sample material is collected with the capillary sample device and the capillary device is inserted back into the test cartridge before the test cartridge is inserted into the Allegro Analyzer. The Test Cartridge has a barcode label with lot specific information.
Estimated Average Glucose is the average glucose levels for the past 60 to 90 days calculated using the following NGSP[1] recommended formula: eAG (mg/dl) = 28.7 × A1C − 46.7.

[1]Harmonizing Hemoglobin A1c Testing, https://ngsp.org/A1ceAG.asp

The Allegro HbA1c Test Cartridge contains the following:

- Reagent 1 - Lysis solution containing a small concentration of detergent and Azide in water
- Reagent 2 - Polystyrene latex particles in water
- Reagent 3 - Mouse monoclonal antibody in a buffered solution containing salts and Bovine Serum Albumin (BSA)
- Reagent 4 - Goat polyclonal antibody in a buffered solution containing salts and Bovine Albumin (BSA)

The Nova Allegro Analyzer is a compact multi-assay analyzer for point-of-care testing, designed to work with Nova Allegro Test Cartridges. The analyzer supports multiple wavelengths that are used to measure the assay of interest. The analyzer contains two analytical bays where the single-use test cartridges are analyzed. Only the right cartridge bay of the Allegro Analyzer can be used with Allegro HbA1c Test Cartridges. When the HbA1c test cartridge bar code is scanned for analysis, the right bay of the analyzer will open. To support the HbA1c Assay, the instrument has a control feature to ensure for the correct sample type and sample volume.

B Principle of Operation:

The Nova Allegro HbA1c test cartridge measures the total glycated hemoglobin and the total hemoglobin concentration. The ratio between them is proportional to the % HbA1c of the sample. The analyzer calculates the ratio. A whole blood sample containing hemoglobin A1c and total hemoglobin are nonspecifically absorbed to latex particles. The anti-human mouse HbA1c antibody reacts to form a complex. Agglutination occurs when polyclonal antibody specifically reacts with the mouse antibody bound to the hemoglobin A1c on the surface of the latex particles. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a stored calibration curve and displayed on the Nova Allegro Analyzer.

C Instrument Description Information:

1. Instrument Name:

   Nova Allegro Analyzer

2. Specimen Identification:

   Specimen identification can be done by scanning a patient identification bar code or by manual entry.

3. Specimen Sampling and Handling:

   A single-use, disposable capillary sample collection device, which is part of the HbA1c Assay Test Cartridge, is used to obtain 1.5 µL fingerstick capillary whole blood to the test cartridge. The capillary sample collection device containing the blood is inserted into the HbA1c test cartridge, then the test cartridge is placed into the sample bay within 1 minute of sample collection and the start icon is pressed to start analysis.

4. Calibration:

   The reagent test cartridge includes a scannable 2D barcode containing the stored calibration curve. The HbA1c value is obtained using the stored calibration curve and is displayed on the Nova Allegro Analyzer.

5. Quality Control:

   External Quality Control (QC) material is used to monitor the performance of the analyzer and the test cartridges used with the analyzer and to verify the analyzer is performing to specifications. QC recommendations are found in the labeling.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Alere Afinion HbA1c on Afinion 2 analyzer

B Predicate 510(k) Number(s):
K171650

C Comparison with Predicate(s):

Device & Predicate Device(s):	K221326	K171650
Device Trade Name	Nova Allegro HbA1c Assay	Afinion HbA1c
General Device Characteristic Similarities		
Intended Use/Indications For Use	For quantitative determination of glycated hemoglobin (% hemoglobin A1c, % HbA1c).	Same
General Device Characteristic Differences		
Test Principle	Latex enhanced turbidimetric immunoassay	Automated boronate affinity assay

Device & Predicate Device(s):	K221326	K171650
Device Trade Name	Nova Allegro Analyzer	Afinion 2 analyzer
General Device Characteristic Similarities		
Intended Use/Indications For Use	For in vitro diagnostic use for the quantitative determination of assays using test cartridges.	Same
General Device Characteristic Differences		
Cartridge Interface	Physical processing of cartridge (locking, docking, foil penetration, mixing assay)	Physical processing of cartridge (locking, docking, splitting, foil penetration, merging)
Peripheral units	Barcode reader and printer integrated into analyzer	A barcode reader and printer can be connected

VI Standards/Guidance Documents Referenced:

None referenced

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision studies were performed for the Nova Allegro HbA1c Assay by operators who are representative of intended CLIA waived users at four point of care (POC) sites representative of CLIA waived settings using a total of eight Nova Biomedical Allegro Analyzers (2 per site) and three lots of Allegro HbA1c Test Cartridges.

20-Day Imprecision (Controls)
Two Nova Allegro HbA1c Control Solutions were analyzed for twenty days in duplicate twice a day (total of eighty measurements per sample) at each of the four POC CLIA waived sites, using three HbA1c test cartridge lots and three different operators who are representative of intended CLIA waived users at each site. Analyses were performed for each individual site and for all sites combined and are presented below:

		Repeatability		Between-Run/Operator		Between-Day/Lot		Between-Site		Reproducibility	
	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Control 1	5.67	0.091	1.61%	0.037	0.65%	0.034	0.60%	0.045	0.79%	0.113	2.00%
Control 2	9.47	0.175	1.85%	0.035	0.37%	0.118	1.25%	0.112	1.18%	0.242	2.55%

5-Day Imprecision (Venous Whole Blood)

The sponsor provided a precision study using venous whole blood, a validated surrogate for the intended use sample. The sponsor additionally provided data to support that venous blood samples did not behave differently from capillary whole blood samples. Three K2EDTA venous whole blood samples were analyzed for five days in duplicate four times a day (total of forty measurements per sample) at four sites, using three different HbA1c test cartridge lots and three different operators at each site. Each site used its own venous whole blood samples; therefore, only individual site analysis was performed for the blood samples and are shown below:

Venous Whole Blood Samples	Mean	Repeatability		Between-Run/Operator		Between-Day		Between-Lot		Within-Laboratory	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Blood 1	6.68	0.110	1.65%	0.045	0.68%	0.041	0.61%	0.049	0.73%	0.126	1.89%
Blood 2	7.99	0.093	1.16%	0.077	0.96%	0.029	0.36%	0.032	0.40%	0.124	1.55%
Blood 3	12.25	0.273	2.23%	0.142	1.16%	0.125	1.02%	0.129	1.05%	0.332	2.71%

Repeatability (Capillary Fingerstick Blood)

For evaluation of fingerstick precision/repeatability, a second fingerstick specimen was collected and measured for HbA1c on 524 subjects enrolled in the Method Comparison study. Three lots of HbAlc test cartridges, eight analyzers (two at each of four sites) and three different operators who are representative of intended CLIA waived users conducted the fingerstick precision measurements. Estimates of repeatability are shown below.

			Repeatability	
HbA1C range	N	Mean	SD	%CV
4.0 - 6.0	200	5.50	0.083	1.50%
6.1 – 8.0	169	6.83	0.103	1.51%
8.1 – 10.0	106	8.94	0.171	1.92%
10.1 – 14.0	49	11.32	0.270	2.38%

Reproducibility (Total Imprecision) Estimate

Total imprecision including repeatability, between-run/operator, between day/lot and between-site is shown below.

HbA1c range	FS Repeatability	VWB Between-Run/Operator	VWB Between-Day/Lot	QC Between-Site	Reproducibility	
	SD	SD	SD	SD	SD	%CV
4.0 - 6.0	0.083	0.045	0.049	0.045	0.115	2.31%
6.1 -8.0	0.103	0.045	0.049	0.045	0.131	1.87%
8.1 – 10.0	0.171	0.077	0.032	0.112	0.221	2.45%
10.1 -14.0	0.270	0.142	0.129	0.112	0.349	2.91%

FS -fingerstick; VWB- venous whole blood

2. Linearity:

A study was performed using 11 whole blood samples with HbA1c concentrations spanning 4.0% to 15.4%. The 11 samples were created by mixing varying aliquots of a low and a high concentration sample. Each sample was assayed in replicates of four on one Nova Allegro Analyzer using one lot of Allegro HbA1c Test Cartridges and the mean of replicates was compared to results obtained on the Tosoh G8 analyzer. The results from linear regression results are shown below:

Analyte	Range Tested (%)	Levels	Slope	Intercept	r
HbA1c	4.0 - 15.4	11	1.016	-0.14	0.999

All deviations from linearity were within ±5%. The results of the linearity study support the sponsor's claim that the Nova Allegro HbA1c Assay on the Nova Allegro Analyzer is linear across the measuring range of 4.0 -14.0 % HbA1c. If a test result is outside the Allegro's measuring range, the analyzer will not display a HbA1c result. The analyzer screen shows a red bar graph flag and <4.0% or >14.0% are displayed. The analyzer printout shows 3 down arrows or 3 up arrows and <4.0% or >14.0% are displayed on the printout. This function was validated and demonstrated to function as intended.

3. Analytical Specificity/Interference:

Studies were performed to evaluate the impact of common endogenous and exogenous substances in the intended use population on Nova Allegro HbA1c Assay results. Whole blood samples containing HbA1c at two levels (5.4 - 6.6% HbA1c and 8.6 – 10.3% HbA1c) were split into test samples containing potential interfering substances and control specimens without the interfering substance. Test samples were assayed in replicates of 10 and the mean concentration for each test sample was compared to the mean concentration of 10 replicates of the control sample. Two Nova Allegro Analyzers and one lot of Nova Allegro HbA1c Test Cartridges were used. The highest tested concentrations at which no significant interference was observed (defined by the sponsor as less than ±10% difference between the test and control samples) are presented in the following table:

Substances	Highest concentration tested without significant interference
Acetylsalicylic Acid	65.2 mg/dL
Ibuprofen	50.0 mg/dL
Glyburide	200 mg/dL
Cholesterol	1055 mg/dL
Acetaminophen	20.0 mg/dL
Non-conjugated Bilirubin	60.0 mg/dL
Conjugated Bilirubin	30.0 mg/dL
Ascorbic Acid	6.0 mg/dL
Metformin	4.0 mg/dL
Glucose	1000 mg/dL
Glipizide	0.2 mg/dL
Chlorpropamide	74.8 mg/dL
Tolbutamide	64 mg/dL
Acarbose	60 mg/dL
Captopril	0.6 mg/dL
Rheumatoid Factor	572 IU/ml
Ampicillin	100 mg/dL
Cyclosporine	0.5 mg/dL
Heparin	5 U/mL
Levodopa	2 mg/dL
Metronidazole	20 mg/dL
Phenylbutazone	40 mg/dL
Rifampicin	6.4 mg/dL
Theophylline	10 mg/dL
Vitamin B12	1.2 mg/dL
Furosemide	7 mg/dL
Gemfibrozil	8 mg/dL
Losartan	6 mg/dL
Nicotinic Acid	70 mg/dL
Urea	700 mg/dL
y-Tocopherol	10 mg/dL
Atorvastatin	5 mg/dL
Intralipid	1000 mg/dL
Propranolol	0.3 mg/dL
Uric Acid	30.0 mg/dL
Acetylcysteine	166 mg/dL
Cefoxitin	650 mg/dL
Doxycyclin	5 mg/dL
Immunoglobulin	2 g/dL
Methyldopa	2.25 mg/dL
Ozempic (Semaglutide)	0.192 mg/dL
Protein (Total)	21000 mg/dL
Salicylic acid	59 mg/dL
Triglycerides	1520 mg/dL

Hemoglobin Interference

A study was performed to test the impact of hemoglobin on the Nova Allegro HbA1c Assay using six (6) Allegro Analyzers and one (1) lot of reagents. Fifteen venous whole blood specimens with HbA1c concentrations from 5.0 to 11.0 % and hemoglobin concentrations from 10.8 to 14.6 g/dL were prepared to contain hemoglobin at target levels of 5.5 and 20.5 g/dL. The average of three (3) replicates of each blood sample was compared to the average neat HbAlc sample result. The results demonstrated that no significant interference (defined by the sponsor as less than ±10% interference between the test and control samples) was observed from total hemoglobin levels between 6 and 20 g/dL. The Allegro Analyzer will not generate HbA1c results with samples containing hemoglobin concentrations outside of 6.0 - 20.0 mg/dL and will display the error code "HbA1c Bad Sample". This feature was validated and was shown to function as intended.

Cross-Reactivity

Potential cross reactivity from HbA0, HbA1a and HbA1b was evaluated using 226 samples with HbA1c concentrations ranging from 4.9-12.2% and containing HbA0 (83.8-93.5%), HbA1a (0.3-1.6%), HbA1b (0.4-1.7%). Samples were tested using the Allegro Analyzer and results compared to measurements obtained on the Tosoh G8 analyzer.
Results are summarized below.

Potential Interferent	**Highest Level Tested with No Significant Interference**
HbA0	Up to 93.5%
HbA1a	Up to 1.6%
HbA1b	Up to 1.7%

Hemoglobin Derivatives

Potential interference from hemoglobin derivatives with the Nova Allegro HbA1c Assay was evaluated. Two Nova Allegro Analyzers and one lot of Nova Allegro HbA1c Test Cartridges were used to assess venous whole blood specimens at target levels of 5.5 and 8.0 % HbA1c. Each blood sample was divided into a control sample and a test sample, and each was assessed in replicates of 10. Glycated albumin samples were derived with glycated human albumin. Acetylated hemoglobin was derived in the presence of acetylsalicyclic acid, carbamylated hemoglobin was derived in the presence of sodium cyanate, and labile A1c was derived in the presence of glucose. The sponsor defined no-significant interference as ≤±10% difference between the test and control samples and concluded that the following do not interfere with the Nova Allegro HbA1c assay: 50 mg/mL glycated albumin, 50 mg/mL acetylated hemoglobin, 0.5 mg/mL carbamylated hemoglobin, 20 mg/mL labile hemoglobin.

Hemoglobin variant interference

A study was performed to assess potential interference from hemoglobin variants (HbA2, HbC, HbD, HbE, HbS, and HbF) on the Nova HbA1c Assay using two Nova Allegro Analyzers and one lot of Nova Allegro HbA1c Test Cartridges. One hundred and nineteen

(119) venous whole blood samples were measured in this study, 20 samples for each HbC, HbD, HbE and HbF, 25 samples for HbS, and 14 samples for HbA2. Samples were measured on the Nova Allegro Analyzer and compared to results obtained by an FDA cleared comparator method that has been demonstrated to be free from the hemoglobin variant interference. The following tables summarize the samples and study results:

Hemoglobin Variant	Number of Samples Tested	% Content of Variant in Sample	Range in Concentration in % HbA1c
HbC	20	26.3 to 38.2	5.7 to 11.5
HbE	20	20.1 to 27.9	4.9 to 9.9
HbD	20	25.9 to 41.1	5.5 to 13.4
HbA2	14	4.4 to 6.5	5.1 to 9.8
HbS	25	27.2 to 38.6	5.0 to 12.3
HbF	20	3.5 to 54.5	4.7 to 8.3

Hemoglobin Variant	Mean % Bias	Range of relative bias
HbC	1.9%	-6.3% to 10%
HbE	4.4%	-4.3% to 8.3%
HbD	3.0%	-4.7% to 9.5%
HbA2	0.6%	-6.8% to 7.1%
HbS	-4.5%	-9.62% to 6%
HbF	5.4% HbF is the highest HbF concentration where no significant interference (≤ 10%) is observed	

The results from the Nova Allegro HbA1c Assay show that there is no significant interference for samples containing Hemoglobin C (≤ 38.2%), Hemoglobin D (≤41.1%), Hemoglobin E (≤27.9%), Hemoglobin S (≤ 38.6%), and Hemoglobin A2 (≤ 6.5%).

The following warning is included in the Nova Allegro HbA1c Assay package insert:

> This device has significant negative interference with Fetal Hemoglobin (HbF). HbA1c results are invalid for patients with abnormal amounts of HbF, including those with known Hereditary Persistence of Fetal Hemoglobin.

4. Assay Reportable Range:

The claimed measuring range is 4 -14% HbA1c.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Traceability:
The Allegro HbA1c assay on the Allegro Analyzer is traceable to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Reference Method for Measurement of HbA1c. The system is certified with the National Glycohemoglobin

Standardization Program (NGSP). The certification expires in one year. See the NGSP website for current certification at http://www.ngsp.org.

6. Detection Limit:

   The claimed measuring range of 4-14% HbA1c is based on linearity, accuracy and precision.

7. Assay Cut-Off:

   Not applicable.

8. Accuracy (Instrument):

   See Section VII.C.3. Other Clinical Supportive Data.

9. Carry-Over:

   The Nova Allegro HbA1c Test Cartridges are fully self-contained test cartridges. The sponsor describes that no contact is made between the system instrumentation and the reagents or test sample.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   See Section VII.C.3. Other Clinical Supportive Data.

2. Matrix Comparison:

   The Nova Allegro HbA1c Assay on the Nova Allegro Analyzer is for use only with capillary whole blood obtained from the fingertip.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

   Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   The performance of the Nova Allegro HbAlc Assay on the Nova Allegro Analyzer was assessed in the hands of 15 operators who were representative of intended CLIA waived users, at four clinical sites (physician offices). HbA1c results obtained from capillary fingerstick samples from 533 patients were compared to HbA1c results obtained from

matched K2EDTA venous whole blood samples on an FDA cleared comparator method run at a NGSP secondary reference laboratory. A total of eight Allegro Analyzers (2 per site) and three lots of Allegro HbA1c Test Cartridges were used in the study. A Passing-Bablok regression analysis was performed using 526 paired results. Seven results were out of the analytical measuring range and were excluded from the regression analysis. The Passing-Bablok regression results are shown below:

Site	N	Sample Range Allegro (%)	Slope	Intercept	r
1	156	4.7 - 12.9	0.968	0.261	0.993
2	154	4.6 - 13.2	0.974	0.204	0.993
3	102	5.0 - 13.1	0.983	0.180	0.994
4	114	4.4 - 13.8	0.968	0.203	0.994
Combined	526	4.4 - 13.8	0.972	0.217	0.993

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The labeling states the following.

In 2023, the American Diabetes Association (ADA) recommended a reasonable A1c goal for many non-pregnant adults is < 7% (53 mmol/mol) without significant hypoglycemia. On the basis of health care professional judgment and patient preference, achievement of lower A1c levels than the goal of 7% may be acceptable and even beneficial if it can be achieved safely without significant hypoglycemia or other adverse effects of treatment. Less stringent A1c goals (such as <8% [64 mmol/mol]) may be appropriate for patients with limited life expectancy or where the harms of treatment are greater than the benefits. Health care professionals should consider deintensification of therapy if appropriate to reduce the risk of hypoglycemia in patients with inappropriate stringent A1c targets.[1]

[1]American Diabetes Association, Glycemic Targets: Standards of Care in Diabetes-2023, Diabetes Care 2023;46 (Suppl. 1): S97–S110.

F Other Supportive Instrument Performance Characteristics Data:

Electrical safety and electromagnetic compatibility (EMC) testing had been performed and the system was found to be compliant.

Software and cybersecurity documentation was reviewed and found to be acceptable.

The Nova Allegro HbA1c Assay on the Nova Allegro Analyzer is not impacted by altitudes up to 12,000 feet/3650 meters.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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