510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K221508

B Applicant

Sanguina, Inc.

C Proprietary and Established Names

AnemoCheck Home

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
KHG	Class II	21 CFR 864.7500 - Whole Blood Hemoglobin Assays	HE - Hematology

II Submission/Device Overview:

A Purpose for Submission:

Clearance of a new device

B Measurand:

Hemoglobin

C Type of Test:

Semi-quantitative, colorimetric measurement of hemoglobin

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

AnemoCheck Home is intended for home use for the determination of hemoglobin level in whole blood from a finger stick by people over the age of 18. This device is intended for people with anemia caused by iron deficiency anemia, vitamin B12 deficiency anemia, folate deficiency anemia, or who have chronic anemia due to sickle cell disease or thalassemia. AnemoCheck Home tests are for invitro diagnostic use only. Prescription use only.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

None

IV Device/System Characteristics:

A Device Description:

AnemoCheck Home is a semi-quantitative, colorimetric, single-use assay for hemoglobin level determination for prescription home use. AnemoCheck Home device consists of test body and test cap with custom injection molded plastic parts that hold the components of the test. The test cap holds the control material which is embedded in the cap and the blood collection tube. The test body holds the AnemoCheck Home two test vials. These components are placed in the test cap and body during manufacturing.

B Principle of Operation:

The assay principle of AnemoCheck Home is based on the reaction among viable hemoglobin, hydrogen peroxide, and 3,3′, 5,5′-tetramethylbenzidine (TMB). When blood is mixed with the pre-filled solution, an oxidation-reduction (redox) reaction occurs between TMB and hydrogen peroxide, leading to stable oxidized TMB products. The products exhibit different colors based on the amount of total hemoglobin present in the sample. After 2 minutes, the resulting color of the solution allows for visual interpretation with the naked eye by comparing to the provided AnemoCheck Home Color card.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Hemocue Hemoglobin 201 + System

B Predicate 510(k) Number(s):

K032203

C Comparison with Predicate(s):

Device & Predicate Device(s):	K221508	K032203
Device Trade Name	AnemoCheck Home	HemoCue Hb 201 + System

Device & Predicate Device(s):	K221508	K032203
General Device Characteristic Similarities		
Intended Use/Indications For Use	AnemoCheck Home is intended for home use for the determination of hemoglobin level in whole blood from a finger stick by people over the age of 18. This device is intended for people with anemia caused by iron deficiency anemia, vitamin B12 deficiency anemia, folate deficiency anemia, or who have chronic anemia due to sickle cell disease or thalassemia. AnemoCheck Home tests are for in vitro diagnostic use only. Prescription use only.	Quantitative determination of hemoglobin in capillary, venous and arterial whole blood, using a specially designed analyzer, the HemoCue® Hb 201 Analyzer, and specially designed microcuvettes, the HemoCue® Hb 201 microcuvettes. HemoCue® Hb 201 microcuvettes are for In Vitro Diagnostic Use only. The HemoCue® Hb 201 Analyzer is only to be used with HemoCue® Hb 201 Microcuvettes.
Test Principle	Colorimetric oxidation-reduction chemical reaction with hemoglobin	Same
Analyte	Hemoglobin (g/dL)	Same
Storage Conditions	15–30ºC	Same
Single Use	Yes	Same
General Device Characteristic Differences		
Result	Semi-Quantitative	Quantitative
Read out	Manual (Visual-based)	Automated (Reader-based)
Test	Disposable test system	Disposable cuvette
Reportable Range	8–18 g/dL	0–25 g/dL
Sample Volume	5µL	10 µL
Development Time	Rapid (2 minutes)	Rapid (~1 minute time to read)
Reagents	Chemical Reagent in test vial • 3,3',5,5'-tetramethylbenzidine (TMB) (0.60–0.62%) • Hydrogen Peroxide (0.60–0.62%)	Microcuvette
Test Kit	Includes alcohol swabs, gauze pads, adhesive bandages, and disposable safety lancets	Not applicable
Quality Control	Built-in and automatic quality control with an Indicator	External low, mid and high Whole Blood Hemoglobin

Device & Predicate Device(s):	K221508	K032203
	solution, to ensure that AnemoCheck test solution is working and test results are valid.	Controls

VI Standards/Guidance Documents Referenced:

ISO 14971 Second Edition 2007-03-01, Medical devices - Application of Risk Management to Medical Devices
CLSI EP05-A3; Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
CLSI EP25-A; Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision:

Repeatability:
A repeatability study was conducted using four native samples at one external testing site. Samples were tested by three operators with no prior experience testing samples in replicates of 10 on three different lots of AnemoCheck tests. Repeatability results were within the defined acceptance criteria.

			Repeatability		Between Lots		Between Operators		Total	
Sample	**N**	**Mean** (g/dL)	**SD** (g/dL)	**%CV**	**SD** (g/dL)	**%CV**	**SD** (g/dL)	**%CV**	**SD** (g/dL)	**%CV**
3031	90	8.0	0.11	1.31	0.14	1.79	0.14	1.75	0.23	2.82
3034	90	10.0	0.05	0.53	0.21	2.09	0.21	2.09	0.30	3.00
3035	90	11.9	0.33	2.80	0.23	1.92	0.23	1.90	0.46	3.89
3037	90	14.9	0.08	0.56	0.18	1.19	0.17	1.17	0.26	1.76

Reproducibility
A reproducibility study was conducted at three sites by two operators with no prior experience at each site over five days with one run per day. One level of quality control material (K842017, high level) were tested in replicates of 10 on three different lots of AnemoCheck tests. The results are within the predefined acceptance criteria.

			Repeatability		Between Day		Between Lots		Between Operator		Between Sites		Total	
QC	N	Mean (g/dL)	SD	%CV	SD	% CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
High	900	15.1	0.30	2.01	0.33	2.18	0.33	2.19	0.33	2.22	0.33	2.19	0.47	3.09

Lay-User Precision Study

A precision study was performed to evaluate the performance of AnemoCheck by lay users. 13 lay people (5 males, ages 22–44 and 8 females, ages 19–64) were enrolled and provided the device and the instructions for use. Each user tested themselves 10 times with AnemoCheck Home tests. Data analysis was performed to characterize intra-user precision. The results are within the predefined acceptance criteria.

Mean (g/dL)	Interval (g/dL)	SD (g/dL)	% CV
8.9	8.0–10.0	0.2	2.4
11.0	10.1–12.0	0.4	3.6
12.9	12.1–14.0	0.3	2.5
15.9	14.1–18.0	0.4	2.4

2. Linearity:
   Refer to K163215.

3. Analytical Specificity/Interference:
   An interference study was conducted on common exogenous and endogenous substances on AnemoCheck Home tests. Each potentially interfering substance was spiked into four blood samples at or near: 8.0 g/dL, 10.0 g/dL, 12.0 g/dL and a high level (between 14–17 g/dL). Samples were tested in three replicates on one lot of AnemoCheck Home test. Visual interpretation of results should correlate to the correct color block on the color card compared to the control sample result. Potential interfering substances at the concentration in the table below do not interfere with the AnemoCheck test. Refer to K163215 for additional substances.

Substance	Maximum Concentration Tested with no Interference
Endogenous	
High white blood cell High neutrophil count	10,000/µL WBC 6,000/µL neutrophils
Sickle cell - hemoglobin S	15%
Sickle cell - "lyse- resistant"	5%
Folic acid/folate	650 ng/mL
Vitamin B12	900 pg/mL
Iron	200 mg/dL
Ferritin	1000 ng/mL
Transferrin	400 mg/dL
Erythropoietin (EPO)	20 pmol/L
Exogenous	

Substance	Maximum Concentration Tested with no Interference
Hydroxyurea (Hydrea)	0.2 mmol/L
Voxelotor (Oxbryta)	0.4 mg/mL
Deferiprone	0.4 mg/mL
Deferasirox	0.4 mg/mL
Hand sanitizer	5%
Hand lotion	5%

4. Assay Reportable Range:
   The reportable range is 8–18 g/dL.

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

   Transport Stability
   Three different AnemoCheck Home reagent lots were tested after being exposed to different transport conditions which included vibration testing for various time frames (1 day and 3 days), drop testing, extreme temperatures (37°C, 55°C, 4°C, and -20°C) and prolonged high humidity (90% relative humidity for 3 days). Three levels of patient whole blood samples (8.0, 10.0, and 12.0 g/dL) were used to evaluate the AnemoCheck test kits exposed to the different transport conditions. Acceptance criteria were met for all conditions with the exception of studies conducted for the test stored at -20°C. The labeling adequately addresses the limitation that AnemoCheck tests should not be stored frozen.

   Real Time Shelf Life
   In order to establish the shelf life of AnemoCheck Home under specified storage conditions, a shelf life study was conducted according to CLSI EP 25-A. Three AnemoCheck Home reagent lots were tested at several time points (0 (T0), 3, 6, 9, 12, 15, 18, and 21 months) to characterize reagent drift (measurand drift) over time in normal storage (room temperature) conditions using whole blood samples at approximately 8.0, 10.0, and 12.0 g/dL. Three replicates for each test condition, for a total of 36 data points per test condition were tested at different time points and compared to the results at T0. Real time shelf life studies are ongoing and to date, show no indication of measurand drift at time points up to 21 months. The current data supports shelf life claims of 18 months from manufacture date.

   Hemolysate Control Degradation
   In order to assess the degradation of the hemolysate control embedded in the cap of the AnemoCheck Home, three lots of AnemoCheck Home test control material were exposed to high heat (55ºC and relative humidity range of 90–100%) over 12 weeks. Control material was tested at four time points after exposure (t=0 (no exposure, control testing), 4, 8, and 12 weeks) by reconstituting the hemolysate with de-ionized water and testing the hemolysate on a Beckman Coulter DxH 520 Hematology analyzer (K181475) to characterize the hemoglobin level of the control after exposure to high heat and humidity. One trained operator performed ten replicates on each lot tested for each time point. The control result color was determined using the AnemoCheck Home color scale and compared to the control results at time zero with no exposure. Results indicated the hemolysate control material is stable when exposed to high heat over 12 weeks.

AnemoCheck Home Control Degradation

In order to assess the degradation of the hemolysate control as part of the AnemoCheck Home quality control system, three lots of AnemoCheck Home test were exposed to high heat (55ºC and relative humidity range of 90–100%) over 12 weeks. AnemoCheck Home tests were tested at four time points after exposure (t=0 (no exposure, control testing), 4, 8, and 12 weeks) by reconstituting the hemolysate (by adding de-ionized water) and testing the hemolysate on a Beckman Coulter DxH 520 Hematology analyzer (K181475) to characterize the hemoglobin level of the control after exposure to high heat and humidity. One trained operator performed ten replicates on each lot tested for each time point. The control result color was determined using the AnemoCheck Home color scale and compared to the control results. Results indicated the hemolysate control as part of the AnemoCheck Home quality control system, appropriately degrades overtime when exposed to high heat over 12 weeks verifying the internal quality system.

Control Degradation Color Range Study

In order to characterize the color results of normal and degraded control results in AnemoCheck Home tests, a control degradation color range study was conducted. Three lots of AnemoCheck Home tests were degraded to different degrees and tested on freshly prepared blood samples. On the day of testing, venous blood was collected from a healthy volunteer and was mechanically split into plasma and red blood cells (RBCs). Plasma and RBCs were combined in different ratios to create blood samples with the following Hgb values confirmed on the Beckman Coulter DxH 520 Hematology analyzer (K181475): 8.2, 10.3, 12.7 and 15.6 g/dL. Each sample was tested in triplicate across three lots of AnemoCheck Home tests for each condition listed: no test degradation, mild test degradation (within the green definition, 9.0 g/dL green), and moderate test degradation (borderline yellow, 10.0+ g/dL yellow). Results indicate the color results appropriately indicate when control degradation has occurred to ensure the quality control system metrics.

6. Detection Limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) studies were performed using three different lots of AnemoCheck Home tests. Detection limit studies were performed with fresh venous blood from a healthy volunteer. Blood was mechanically split into plasma and red blood cells and combined in different ratios to create blood samples with specific hemoglobin (Hgb) values. All sample values were confirmed on the HemoCue Hb 201+ System (K032203). Studies were performed by one trained operator per the AnemoCheck Home Instructions for Use on three lots with 10 replicates per lot, per sample.

For the LoB and LoD study, four low hemoglobin samples were tested 0.0, 0.4, 0.7 and 1.4 g/dL. For both studies, the blank 0.0 g/dL did not elicit any visual color change and all other samples elicited a blue result correlating to the lowest color block in the reportable range when interpreted between 2–10 minute development time. The LoB is 0.0 g/dL. The LoD is 0.4 g/dL.

For the LoQ study, each sample produced differentiable results equal to or greater than 8.0 g/dL and less than or equal to 18 g/dL. Results were analyzed via linear regression across all sample results for each lot separately and each lot produced an acceptable linear relationship supporting limits of quantitation of 8.0 g/dL at the lower end of the range and 18.0 g/dL at

the upper end of the range. The LoQ is determined to be 8.0 g/dL and 18.0 g/dL at the lower and upper ends of the operating range, respectively.

7. Assay Cut-Off:
   Not applicable.

B Comparison Studies:

1. Method Comparison:
   A method comparison study was conducted in order to demonstrate substantial equivalence between the candidate device and the predicate, HemoCue 201+ System (K032203) and the reference method, Beckman Coulter DxH 520 Hematology analyzer (K181475). Subjects were provided test kits that included instructions for use and kit components in a private testing room. Subjects performed the first fingerstick using the candidate test according to the instructions for use. This sample was considered the lay user sample. A second fingerstick was collected in a BD Microtainer Brand Tube with EDTA (K2) (K940905) by study staff for predicate and comparator testing after the candidate test was completed. The study was conducted at four testing sites enrolling 83 subjects specifically targeting subjects with anemia or at high risk of anemia including patients with iron deficiency anemia, vitamin B12 deficiency, folate deficiency and anemia due to sickle cell or thalassemia.

	N	**Range (g/dL)**	**(r)***	**Slope (95% CI)**	**Intercept (95% CI)**
Candidate vs Comparator (K181475)	83	8.0–14.4	0.94	1.0 (0.9, 1.0)	0.2 (-0.5, 0.9)
Candidate vs Predicate (K032203)	83	8.0–14.8	0.94	1.0 (0.9, 1.1)	-0.1 (-1.1, 0.8)

*Pearson's Correlation

2. Matrix Comparison:
   Not applicable

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):
   Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Refer to K163215.

F Other Supportive Instrument Performance Characteristics Data:

1. Usability Study:

   A usability study was conducted by enrolling participants in the study who were representative of the intended users of each condition (iron deficiency anemia, vitamin B12 deficiency anemia, folate deficiency anemia, or anemia due to sickle cell or thalassemia). Results demonstrated that users were able to perform all critical tasks to complete a successful self-test.

2. Flex Studies:

   The following Flex Studies were performed at one internal site by a single trained operator. A venous blood donation was collected into EDTA anticoagulant tubes and then mechanically split into plasma and red blood cells (RBCs). Plasma and RBCs were then combined in different ratios to create blood samples with the following Hgb values: 8.0, 10.0 and 12.0 g/dL. Hgb values were determined by the Beckman Coulter DxH 520 Hematology Instrument (K181475). Testing was performed in triplicate and on three lots of AnemoCheck Home tests for each condition. Each replicate was analyzed against the control sample level result. All flex studies meet the predefined acceptance criteria with the exception of underfilling, overfilling by 40% and no mixing. The labeling reflects these results.

   Overfill Sample Study

   A flex study was performed testing the "overfill" or when a larger than expected blood sample (6 µL and 7 µL sample volume, or 20% and 40%, respectively) is delivered to an AnemoCheck Home test. Overfilling by 20% or a total of 6 µL led to acceptable results. Overfilling by 40% or a total of 7 µL led to results that were greater than >1.0 g/dL higher than control results. This condition results in higher than expected hemoglobin levels. Overfilling by 40% is obvious upon visual inspection as excessive blood would be present on the test cap. Labeling reflects and informs the user that using the test with excess blood on the cap may lead to inaccurate results.

   Underfill Sample Study

   A flex study was performed to evaluate device performance when the calibrated capillary tube is underfilled by 20% less or 4 µL sample volume. Insufficient volume led to results that were >1.0 g/dL lower compared to control results. This condition results in lower than expected hemoglobin levels. Underfilling is obvious upon visual inspection of the blood collection tube. Labeling reflects and informs the user that using the test with insufficient blood in the capillary tube may lead to inaccurate results.

   Sample delivery time

A flex study was performed to evaluate the device performance when testing is delayed five minutes after sample collection. This study is to characterize the effect of the time delay allowed before sample addition and if accurate test results can be obtained. After sample collection into sample collection tube, the sample was delayed to the insertion of the test body for five minutes. All replicates were within 1.0 g/dL compared to the control sample results. The effect of prolonged waiting in sample tube is mitigated by the presence of coated anticoagulant on the insides of the sample collection tube.

Undermixed test
A flex study was performed to evaluate the device performance when the sample is not mixed and undermixed (3 seconds of mixing). Mixing drives the removal of sample from capillary tube and the end-user is instructed to mix for 5–10 seconds. This study is to characterize the mixing time required for sample addition and control testing. No mixing yielded all invalid results (no control color present). Undermixing (3 seconds of mixing) the sample yields acceptable results compared to the control test. Labeling reflects and informs the user that undermixing can lead to inaccurate results.

Incorrect development time
A flex study was performed to evaluate the device performance when the control and test are read at different times. Control and tests are instructed to be read after 2 minutes and before 10 minutes. Control and test results were read at 1.5, 3 minutes, 5 minutes, and 10 minutes. All replicates were within 1.0 g/dL compared to the control sample results for all times tested. Labeling reflects the appropriate development time to the end-user.

Environmental Factors Flex Studies
A flex study was performed to evaluate the device performance under extreme environmental conditions (33℃ and 90% relative humidity). The testing of all replicates was performed under the extreme conditions from start to finish. All replicates were within 1.0 g/dL compared to the control sample results and acceptable.

Open Vial Stability
A flex study was performed to evaluate the device performance if the screw caps were remove from the test body and the vials of the test body were exposed to air for 5 minutes and 10 minutes. The end-user is instructed to remove the screws caps and immediately insert the test cap into the test body. All replicates were within the 1.0 g/dL compared to the control sample results and acceptable.

Dim/Dark lighting condition test read
A flex study was performed to evaluate the ability to read and interpret the color scales on the device under low/dim lighting (~14 FC or ~150 LUX). All replicates were within 1.0 g/dL compared to the control sample results and acceptable. This study is to characterize if test results can be accurately interpreted under low/dim lighting conditions.

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.