510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K231776

B Applicant

Streck, Inc.

C Proprietary and Established Names

Cell-Free DNA BCT

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QMA	Class II	21 CFR 862.1676 - Blood Collection Device for Cell-Free Nucleic Acids	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

Modification to an existing device

B Measurand:

Not applicable – blood collection device

C Type of Test:

Not applicable

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Cell-Free DNA BCT is a direct-draw venous whole blood collection device intended for collection, stabilization, and transport of venous whole blood samples for use in conjunction with cell-free DNA next generation sequencing liquid biopsy assays that have been cleared or approved for use with samples collected in the Cell-Free DNA BCT device.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

Performance characteristics for this device have only been established on the Guardant360 CDx assay and Guardant Shield assay.

Do not store outside of established conditions.

Do not transfer samples drawn into tubes containing other anti-coagulants and/or preservatives into Cell-Free DNA BCT.

Do not use past expiration date printed on label.

Do not use for clinical chemistry assays or assays other than liquid biopsy next-generation sequencing.

Do not use for collection of materials to be injected into patients.

Cell-Free DNA BCT is not intended for the stabilization of RNA nor is it intended for viral or microbial nucleic acids.

D Special Instrument Requirements:

Not applicable

IV Device/System Characteristics:

A Device Description:

Cell-Free DNA BCT is a sterile, single use, direct-draw blood collection tube comprised of 3 components (i.e., glass tube with rubber stopper, anticoagulant, and cell preservatives). The blood collection tube is a 10mL evacuated tube manufactured with USP Type III glass containing cerium oxide (to prevent color change associated with gamma irradiation sterilization). Each tube includes 200 µL ± 10% of liquid reagent. The reagent composition includes an anticoagulant K_3EDTA and a preservative.

B Principle of Operation:

The device is intended to be placed inside a tube holder or an adaptor that contains a needle designed to pierce the tube closure and allow blood to flow into the tube. Once the vein has been penetrated (using a standard blood collection needle or a blood collection set), the tube is pushed into the holder, and the blood enters the tube. Once a tube has drawn the appropriate amount of blood (10 mL), it is disengaged from the holder and inverted 10 times to mix the reagents with the blood. The specimen is then transported to the lab for plasma isolation and extraction of cfDNA.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Cell-Free DNA BCT

B Predicate 510(k) Number(s):

DEN200001

C Comparison with Predicate(s):

Device & Predicate Device(s):	K231776	DEN200001
Device Trade Name	Cell-Free DNA BCT	Same
General Device Characteristic Similarities		
Intended Use/Indications For Use	Cell-Free DNA BCT is a direct-draw venous whole blood collection device intended for collection, stabilization, and transport of venous whole blood samples for use in conjunction with cell free DNA next-generation sequencing liquid biopsy assays that have been cleared or approved for use with samples collected in the Cell-Free DNA BCT device	Same
Anticoagulant	K_3EDTA	Same
Storage temperature for empty tube	2-30°C	Same
Sample preservation after draw	18°C to 25°C for up to 7 days total, including shipping	Same

Device & Predicate Device(s):	K231776	DEN200001
Nominal draw volume	10 mL	Same
General Device Characteristic Differences		
Assay	Guardant360 CDx Assay and Guardant Shield Assay	Guardant360 CDx Assay

VI Standards/Guidance Documents Referenced:

ISO 11137-1; 2015: Sterilization of health care products-Radiation: Part 1

ISO 11137-2: 2013 Sterilization of Health Care Products-Radiation- Part 2. Establishing the Radiation Dose

CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Repeatability
Within-lot (between tube) variability was evaluated using native venous whole blood samples collected from 59 healthy donors into six BCTs from a single Cell Free DNA BCT manufacturing lot. For each donor, the six BCTs were processed into three cfDNA replicates. Plasma was isolated from samples within 7 days of collection. The isolated plasma was then shipped to Guardant Health (GH) on dry ice and stored at -80°C until further processing. cfDNA was extracted and sequenced on the NovaSeq 6000 Sequencing System. Of the 176 samples processed, 162 samples passed quality control metrics and were assessed for concordance. Additional data from a second study was analyzed to evaluate within lot variability in minimally manipulated colorectal cancer (CRC) samples (samples generated by combining a healthy donor sample with a clinical CRC patient plasma) from 29 donors. Three BCTs per donor from a single BCT lot were analyzed for repeatability. Specimens were shipped to GH and plasma was isolated after receipt. Guardant Shield assay (P230009) results were compared across technical replicates for each sample and assessed for concordance. Variability between replicates for each patient was evaluated based on variant call agreement for somatic variants. A concordant positive call reflects detection of an identical sequencing alteration between replicates, and a discordant call reflects the presence of an alteration in one replicate and the absence of that same alteration in another replicate. Average positive agreement (APA) and average negative agreement (ANA) were calculated as follows:

$$APA = \frac{\#\ concordant\ positives}{\#\ concordant\ positives + \frac{\#\ discordant\ calls}{2}}$$

$$ANA = \frac{\#\ concordant\ negatives}{\#\ concordant\ negatives + \frac{\#\ discordant\ calls}{2}}$$

The results are summarized below:

Endpoint	Healthy	CRC
ANA	95.4%	Not Applicable
APA	60.0%	100%

Reproducibility

Lot-to-lot variability was evaluated using whole blood samples collected from healthy donors and colonoscopy-diagnosed CRC positive donors for the Guardant Shield assay. Samples were drawn into three lots of BCTs (Lot A, Lot B, and Lot C) and these lots were grouped into two conditions (Condition 1 (Lot A vs Lot B) and Condition 2 (Lot A vs Lot C)). The final donor count for samples that were included in the study is as follows: Healthy Donor Condition 1 (Lot A – Lot B) consisted of 28 donors; Healthy Donor Condition 2 (Lot A – Lot C) consisted of 22 donors; CRC Donor Condition 1 (Lot A – Lot B) consisted of 27 donors; and CRC Donor Condition 2 (Lot A – Lot C) consisted of 17 donors. An additional cohort of samples (n=25) were also collected in BCT Lot D and were compared to Lot A (Lot A vs Lot D). All Cell-Free DNA BCTs were processed into plasma within 7 days after whole blood collection, frozen, and shipped for analysis using the Guardant Shield Assay. Variability between Cell-Free DNA BCT lot was evaluated based on variant call agreement for somatic variants. APA and ANA were calculated as described previously. The results are summarized below.

Lot Comparison	ANA	APA
Lot A to B	89.36%	92.06%
Lot A to C	91.30%	87.50%
Lot A to D	95.65%	96.15%

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

Preservative

To validate that the preservative formulation does not interfere with the Guardant Shield assay, venous whole blood samples were collected from healthy and CRC donors. BCTs were manufactured to reflect the normal preservative formulation ("reference"), 2x Preservative A, or 2x Preservative B. Four BCTs (2 reference BCTs and 2 test BCTs at 2x A volume) were collected from 30 healthy donors and 16 CRC donors. Four BCTs (2 reference BCTs and 2 test BCTs at 2x B volume) were collected from 30 healthy donors and 14 CRC donors. Each sample was processed to plasma into individual aliquots, frozen and shipped to Guardant Health on dry ice. One aliquot from each test group and donor was processed for cfDNA extraction and sequencing. cfDNA analysis was conducted using the Guardant Shield assay. The agreement rates were calculated for each BCT preservative condition. The percentages were calculated with the combined set of Healthy and CRC donor samples with respect to the Guardant Shield assay result for the reference condition. Performance was evaluated based on variant call concordance relative to the reference Cell-Free DNA BCT. Each variant called in the reference sample was evaluated in the experimental condition samples by positive percent agreement (PPA). The total number of concordant and discordant calls for all reference positive calls in a given experimental condition was counted across patients and used to calculate PPA. For each reference - treatment sample pair, each eligible site that is negative in the reference sample was assessed for presence of a somatic call in the treatment sample via Negative Percent Agreement (NPA). The total number of concordant and discordant calls for all reference negative calls in a given experimental condition was counted across patients and used to calculate NPA. PPA and NPA were calculated as follows:

$$PPA\ (Experimental+\ |\ Reference+) = \frac{\#\ concordant\ positive\ calls}{\#\ reference\ positive\ calls}$$

$$NPA\ (Experimental-\ |\ Reference-) = \frac{\#\ concordant\ negative\ calls}{\#\ reference\ negative\ calls}$$

The results are summarized below:

Endpoint	2x Preservative A	2x Preservative B
PPA	92.86%	100%
NPA	96.43%	100%

Incomplete Mixing

The instructions for use indicate that the tube should be inverted 10 times after collection. To evaluate the impact of variations in mixing after blood collection, specimens were collected from 30 healthy donors into six BCTs (2 per condition) for the following conditions: 10 inversions (reference condition per the instructions for use), 5 inversions, and 15 inversions. In a supplemental study, samples from 24 CRC donors were collected into four BCT as follows: 2 reference BCTs with 10 inversions and 2 BCTs with either 15 inversions or 5 inversions. Plasma was isolated and frozen into aliquots that were shipped to Guardant

Health for processing. As described above under the Preservative study, PPA and NPA were used to assess variant call concordance. The results indicated that inadequate or overmixing may result in diminished performance.

Healthy	5 inversions	15 inversions
PPA	100.0%	100.0%
NPA	96.5%	100.0%

CRC	5 inversions	15 inversions
PPA	88.89%	100.0%
NPA	66.7%	100.0%

Short Draw
To evaluate potential interference caused by underfilling cfDNA BCTs, four whole blood samples were collected from healthy and CRC donors. BCTs were manufactured to reflect the normal reagent (e.g., preservative and anticoagulant) formulation ("reference"), 2x volume of reagent, or 3.3X reagent. These conditions were intended to reflect 10 mL whole blood collected, 5 mL whole blood collected, and 3 mL whole blood collected. Each sample was processed to plasma in individual aliquots, frozen and shipped to Guardant Health on dry ice. For each donor, plasma isolated from the 2 reference BCTs were pooled and processed into a single assay replicate and plasma isolated from the 2 test BCTs were pooled and processed into another single assay replicate. As described above, PPA and NPA were used to assess variant call concordance.

Endpoint	2x Reagent (5mL whole blood)	3.3x Reagent (3 mL whole blood)
PPA	82.14%	100%
NPA	87.50%	84.62%

Tube Stopper
Extractable and leachable studies were performed to identify substances within the tube stopper that may interact with patient specimens and interfere with the ability of the tube to preserve cfDNA. The results support that extractables from the tube stopper are not anticipated to interfere with device performance.

4. Assay Reportable Range:

   Not applicable.

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

   Shelf-Life
   To assess the impact of age (shelf-life) of Cell-Free DNA BCT on their ability to preserve a sample of whole blood for optimal performance of the Guardant Shield assay, blood was collected from healthy and CRC subjects. Four lots of BCTs were used for each health condition (healthy or CRC). For each donor, samples were collected into two reference lots of BCTs (time 0) and two test lots of BCTs (4 months, 12 months or 18 months old at study initiation). Each sample was processed to plasma, frozen as individual aliquots, and shipped to Guardant Health on dry ice. For each donor, plasma isolated from the two reference lot

BCTs were pooled and processed into a single assay replicate and plasma isolated from the two test lot BCTs were pooled and processed into another single test assay replicate. PPA and NPA values were calculated comparing the reference timepoint (time 0) and the respective test timepoints, 4, 12, and 18 months .The study results support that devices stored for 18 months prior to blood collection are able to maintain cfDNA concentration and integrity for use with the Guardant Shield assay.

Comparison	PPA	NPA
0 to 4	87.88%	95.65%
0 to 12	93.33%	87.50%
0 to 18	96.15%	95.65%

Impact of Tube Storage Temperature Study

To examine the equivalency of Guardant Shield assay outputs to BCT storage temperature and the stability of whole blood stored at room temperature for up to 8 days after blood draw, 60 donors were collected into 12 BCTs across two lots. Before sample collection the BCTs (aged 15 months or greater) were stored at 2°C, 22°C, or 30°C. Once collected, both healthy and minimally manipulated CRC samples were stored for 0 or 8 days at room temperature. NPA and PPA were assessed by comparing binary Guardant Shield Test results between the reference and test condition for healthy donors and minimally manipulated CRC samples. The study results support that pre-aged devices, stored at 2-30°C, are able to maintain cfDNA concentration and integrity for use with the Guardant Shield assay for up to 8 days after blood collection.

Healthy	Day8_22°C Reference vs Day8_2C Test	Day8_22°C Reference vs Day8_30C Test	Day0 Reference vs Day8 Test
ANA	96.4%	96.4%	97.0%
APA	0.0%	50.0%	26.2%

CRC	Day8_22°C Reference vs Day8_2°C Test	Day8_22°C Reference vs Day8_30°C Test	Day0 Reference vs Day8 Test
ANA	Not Applicable	Not Applicable	Not Applicable
APA	100.0%	100.0%	100.0%

Sample Shipping Stability

To evaluate the stability of whole blood specimens collected in cfDNA BCTs for up to 10 days across the expected range of sample transport and storage conditions of the blood collection kits, 130 healthy and 60 positive samples were collected and evaluated. Specimens were shipped overnight to Guardant for processing to plasma. Reference BCTs pertaining to T0 testing were processed on the day of receipt. The remainder of the BCTs for each donor were stored horizontally in their respective blood collection kits until a set timepoint. Post-collection BCTs were treated for 72 hours with extreme summer or winter shipping temperatures (according to ISTA 7E, approximately -10°C to 35°C) and the remaining time at ambient temperature (18-25°C). Plasma was isolated one, six, eight, or ten days after receipt of sample. For samples subjected to extreme winter shipping conditions (potentially to -10°C) and stored for up to 10 days at room temperature, PPA was 100%, and NPA was

100%. For samples subjected to extreme summer shipping conditions (potentially to 35°C) and stored for up to 10 days at room temperature, PPA was 100% and NPA was 96.30%.

Whole Blood Room Temperature
A supplemental study with 15 native CRC samples was performed to demonstrate sample stability for up to 8 days at room temperature. Each donor provided blood samples in four BCTs. Two BCTs were processed to plasma on the day of sample receipt (Day 1 after sample collection) and serve as the reference condition sample (T0). The remaining 2 BCTs were stored in the BCT for 7 days (Day 8 after sample collection) at room temperature before being processed to plasma; this served as the T1 condition sample. NPA and PPA were assessed by comparing binary Guardant Shield assay results between the reference and test condition for the CRC donors.

CRC	Reference (Day 1) vs Condition (Day 8)
NPA	50.0%
PPA	100.0%

Additional Studies
Additional studies were conducted to assess robustness to centrifugation and stopper interference. Study protocols, acceptance criteria, and results for these studies were provided and found to be acceptable.

Stopper closure assembly stability, stopper pullout force, stopper resealing, and anticoagulant effectiveness study performance was established in DEN200001.

6. Detection Limit:

   Not applicable.

7. Assay Cut-Off:

   Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not applicable. Accuracy of results from samples collected in Cell-Free DNA BCT was established within the assay's clinical study. Refer to P230009.

2. Matrix Comparison:

   Not applicable; device is for whole blood only.

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):

   Refer to P230009.

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Not applicable

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.