510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K192947

B Applicant

Ancestry Genomics, Inc.

C Proprietary and Established Names

AncestryDNA Saliva Collection Kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
OYJ	Class II	21 CFR 862.1675 - Blood Specimen Collection Device	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Not applicable

C Type of Test:

Collection and stabilization of genomic DNA from saliva for use in molecular genotyping testing

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The AncestryDNA Saliva Collection Kit is intended for use in the noninvasive collection of saliva samples for in vitro diagnostic testing of human DNA. Saliva may be collected by spitting directly into the AncestryDNA Saliva Collection Kit by a lay user. Saliva samples collected using the AncestryDNA Saliva Collection Kit are stabilized and isolated for use with over-the-counter AncestryDNA Genetic Health Risk Tests. Saliva samples collected using the AncestryDNA Saliva Collection Kit can be transported and/or stored long term at ambient conditions.

C Special Conditions for Use Statement(s):

OTC - Over The Counter
AncestryDNA Saliva Collection Kit is for use with AncestryDNA Factor V Leiden Genetic Health Risk Test.
This kit is intended for users 18 years of age and older.

D Special Instrument Requirements:

None.

IV Device/System Characteristics:

A Device Description:

The AncestryDNA Saliva Collection Kit consists of saliva collection tube, funnel, cap, blister pack, collection bag with an absorbent pad. The cap contains DNA stabilization solution. Saliva is delivered directly by spitting into the collection tube via the funnel. A cap is provided that releases the stabilization solution and closes the tube for transport and storage.

B Principle of Operation:

The AncestryDNA Saliva Collection Kit is used for collecting and stabilizing human DNA from saliva and for the transportation and long-term ambient room temperature storage of a saliva sample. Saliva is delivered directly by expectorating into the collection tube via the funnel. Once the user has provided the saliva sample, the user removes the funnel from the saliva collection tube and affixes the cap. Affixing the cap by screwing on releases the stabilization solution. The Instructions for Use instruct the user to shake the tube for at least five seconds to mix the saliva sample with the stabilization solution. Samples can be immediately processed, transported, or stored for future use. Device and sample integrity are preserved during typical ambient transport and storage conditions for up to 12 months.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Oragene Dx OGD-500.001

B Predicate 510(k) Number(s):

K141410

C Comparison with Predicate(s):

Device & Predicate Device(s):	K192947	K141410
Device Trade Name	AncestryDNA Saliva Collection Kit	Oragene Dx OGD-500.001
General Device Characteristic Similarities		
Intended Use/Indications For Use	Intended for noninvasive collection of saliva samples	Same
Intended Use Population	Over the counter	Same
Sample Source	Saliva	Same
Collection Device Contents	Nucleic acid stabilization solution	Same
Analyte	DNA	Same
General Device Characteristic Differences		
Tests intended for use with the saliva collection device	AncestryDNA Factor V Leiden Genetic Health Risk Test	23andMe PGS Bloom Syndrome Carrier Screening Test

VI Standards/Guidance Documents Referenced:

- CLSI Guideline EP07-A3, Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition
- CLSI Guideline EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition
- CLSI Guideline EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition
- CLSI Guideline EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
- CLSI Guideline EP37 1st Edition, Supplemental Tables for Interference Testing in Clinical Chemistry

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Between-Site Reproducibility

A reproducibility study was performed at 2 laboratory sites. Saliva samples from nine donors of known Factor V Leiden genotypes (3 homozygous common, 3 heterozygous, and 3 homozygous rare) were genotyped in triplicate across three plates per site (a total of 162 genotyping events) to evaluate the reproducibility of the performance of the AncestryDNA Saliva Collection Kit (SCK) when used with the AncestryDNA Factor V Leiden Genetic Health Risk (GHR) Test (see k192944). Samples were accepted or rejected using the quality control (QC) criteria of a sample call rate (SCR) or overall call rate per sample of ≥ 98% across the entire array. At the first site, all donor replicates passed QC for the first genotyping run. At the second site, 14.8% (12/81) of replicates failed first pass QC. All replicates (100%) passed the second genotyping run and yielded 100% concordant results with the expected genotype, determined by bi-directional sequencing.

Within-Run and Within-Laboratory Precision

A within-run precision study was conducted at one site with nine donor saliva samples of known Factor V Leiden genotypes (3 homozygous common, 3 heterozygous, and 3 homozygous rare). Samples were tested using the AncestryDNA Factor V Leiden GHR Test (see k192944) in replicates of five. All forty-five replicates passed QC in the first genotyping run and yielded 100% concordant results with the expected genotype, determined by bi-directional sequencing.

A within-laboratory precision study was conducted with nine donor saliva samples of known Factor V Leiden genotypes (3 homozygous common, 3 heterozygous, and 3 homozygous rare). Samples were tested using the AncestryDNA Factor V Leiden GHR Test across multiple AncestryDNA SCK lots, operator teams and days at two laboratory sites. At the first site, 0.3% (6/1719) of replicates failed first pass QC. Three of these six replicates passed QC after the second genotyping run, leaving a total of 3 out of 1,719 replicates (0.2%) that failed QC. The 1,716 replicates that passed QC yielded 100% concordant results with the expected genotype, determined by bi-directional sequencing. At the second site, 3.2% (20/621) of replicates failed first pass QC. All replicates (100%) passed the second genotyping run and yielded 100% concordant results with the expected genotype, determined by bi-directional sequencing.

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

Effect of Endogenous Interfering Substances:
An endogenous substance interference study was conducted with salivary α-amylase (395 U/ml), hemoglobin (20 mg/mL), immunoglobulin A (IgA) (0.44 mg/mL), and total protein (0.185 mg/mL salivary α-amylase + 0.44 mg/mL IgA + 2.05 mg/mL human serum albumin). Five saliva samples from each of ten (10) donors were pooled, divided into five aliquots, and spiked with one of the four interfering substances or phosphate buffered saline (PBS) for the control sample. Samples were genotyped using the AncestryDNA Factor V Leiden Genetic Health Risk Test (see k192944) in triplicate for a total of 150 genotyping events. All samples were evaluated for the effect of the substance on sample call rate (SCR) and percent agreement with the true variant status, as determined by bi-directional sequencing. Three (3) replicates from one donor required a second genotyping run after failing sample quality control (sample call rate ≥ 98%) on the first genotyping attempt. All specimens spiked with endogenous substances passed the quality control metric and demonstrated 100% concordance for all interfering substances.

Effect of Exogenous Interfering Substances:
Potentially interfering exogenous substances introduced into saliva samples through the following activities were assessed: eating beef, eating chicken, drinking alcohol, chewing gum, using mouthwash, and smoking. Each group of 12 donors ate chicken, drank alcohol, or used mouthwash. Each group of 10 donors ate beef, chewed gum, or smoked. Each donor provided three samples: a baseline/control sample taken at least 2 hours prior to the activity, a sample collected immediately after the activity, and a sample collected 30 minutes after the activity. All samples were evaluated for the effect of the substance on sample call rate and percent agreement with the true variant statues, as determined by bi-directional sequencing.

For all genotyping events where the control samples passed QC, there was 100% agreement between the AncestryDNA Factor V Leiden GHR Test (see k192944) results and bi-directional DNA sequencing for all activities, demonstrating no effect of any interfering substances on genotyping.

For the eating chicken and eating beef activities, a higher rate of QC failures for samples collected immediately after completing the activity compared to matched donor control samples was observed. The AncestryDNA Saliva Collection Kit includes the following sentence as part of the saliva collection warning: “Do NOT eat, drink, smoke, or chew gum for 30 minutes before giving your saliva sample.”

Please refer to k192944 for additional interference study information.

4. Assay Reportable Range:

Not applicable.

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

Pre-collection shelf-life stability

For pre-collection stability studies, the stability of the AncestryDNA SCK was assessed after exposure to multiple storage conditions (i.e., frozen -30˚C to -15˚C, refrigerated 2˚C to 8˚C, or room temperature 15˚C to 30˚C) prior to saliva sample collection and genotyping. For each storage condition, saliva samples from 42 donors with unknown Factor V Leiden genotypes were evaluated using 3 lots of AncestryDNA SCKs. DNA from donor saliva samples was extracted and DNA concentration and purity were determined. Samples were subjected to genotyping using the AncestryDNA Factor V Leiden GHR Test (see k192944) and compared to the true variant status determined by bi-directional sequencing. Stability testing protocols and acceptance criteria were reviewed and found to be acceptable. Current real-time data supports a pre-collection shelf-life stability claim of 12 months at 15˚C to 30˚C.

Post-collection sample stability

For post-collection stability studies, the stability of saliva-filled AncestryDNA SCKs exposed to multiple storage conditions (i.e., frozen -30˚C to -15˚C, refrigerated 2˚C to 8˚C, or room temperature 15˚C to 30˚C) prior to genotyping was assessed. For each storage condition, saliva samples from 42 donors with unknown Factor V Leiden genotypes were evaluated using 3 lots of AncestryDNA SCKs. DNA from donor saliva samples was extracted and DNA concentration and purity were determined. Samples were subjected to genotyping using the AncestryDNA Factor V Leiden GHR Test (see k192944) and compared to the true variant status determined by bi-directional sequencing. Stability testing protocols and acceptance criteria were reviewed and found to be acceptable. Current real-time data supports a post-collection sample stability claim of 12 months at 15˚C to 30˚C.

Transport stability

Simulated transport stability studies were conducted to assess the effect of temperature and humidity extremes on the AncestryDNA SCK. Pre- and post-collection AncestryDNA SCKs were tested according to the ISTA 3A standard. After being subjected to simulated transport conditions, pre-collection AncestryDNA SCKs were assessed for integrity (gross fluid leakage) and the pH of the DNA stabilization solution relative to pH specifications was determined. After being subjected to simulated transport conditions, post-collection AncestryDNA SCKs were assessed for integrity (saliva volume relative to the fill line) and ability to meet the sample call rate of $\geq$ 98% prior to testing with the AncestryDNA Factor V Leiden GHR Test (see k192944). Stability testing protocols and acceptance criteria were reviewed and found to be acceptable. The stability study data support pre-collection and post-collection AncestryDNA SCK transport at temperatures ranging from -29ºC to 50ºC and 15% to 85% relative humidity.

6. Detection Limit:

Sample Volume Tolerance
The AncestryDNA Saliva Collection Kit (SCK) is intended for collection of 1 mL of saliva. A study was conducted to evaluate the effect of over or under filling the AncestryDNA SCK.

Donors provided saliva in three different devices with varying fill-to lines (0.5 mL, 1 mL, and 1.5 mL). The volume of stabilizing liquid in each device was unchanged. The actual amount of saliva collected as well as the impact of delivering less and more saliva on sample call rate (SCR) and genotype testing were examined. A total of 240 DNA samples from 80 donors (60 homozygous common, 15 heterozygous, and 5 homozygous rare) were analyzed for SCR ≥ 98% and were tested with the Ancestry DNA Factor V Leiden Genetic Health Risk (GHR) Test (see k192944). The study was conducted using 1 lot of AncestryDNA SCK and 3 lots of reagent at one site by 8 operators.

The saliva volumes collected were normally distributed around the fill-to lines, with the mean volumes being equal to the fill-to volume plus the 0.9 mL of DNA stabilizing solution in the AncestryDNA SCK. The median amount of saliva collected was typically just above the fill-to line (0.55 mL [0.5 mL target], 1.06 mL [1 mL target], 1.51 mL [1.5 mL target]). The study demonstrated that as the fill-to line increases to "over-fill" at 1.5 mL, the quality control (QC) failure rate (SCR ≥ 98%) also increases (see table below). Nine (9) out of 240 samples failed sample call rate QC. The QC failures were not donor-specific. Seven (7) of the 9 failed samples were from the over-filled tubes, and 8 of the 9 failed samples were from donors with the wild type genotype. The point estimates of overall percent agreement between bi-directional sequencing and AncestryDNA Factor V Leiden GHR Test showed 100% agreement across all fill volumes and genotypes evaluated for all samples that passed the quality control criteria of ≥ 98% (see table below). The labeling includes the statement "Do not overfill" in the instructions for use to minimize the downstream impact to sample processing.

Fill to line (mL)	**Range of collected sample volume (mL)**	**Median collected sample volume (mL)**	**Samples tested**	**FQC**	**Correct Calls**	**Incorrect Calls**	**No-Calls**	**% Agreement**
0.5 (Underfill)	0.45–2.19	0.55	80	1*	79	0	0	100
1 (Control)	1.43–2.84	1.06	80	1	79	0	0	100
1.5 (Overfill)	1.63–3.71	1.51	80	7	73	0	0	100

*This QC failure occurred in a sample with sample volume (saliva + stabilizing solution) of 2.1mL and is, therefore, not attributable to low sample volume.

7. Assay Cut-Off:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   A method comparison study was performed to determine the accuracy of the genotype obtained for 209 saliva samples collected from unique donors using the Ancestry DNA Saliva Collection Kit and tested with the AncestryDNA Factor V Leiden GHR Test (see k192944) compared to bi-directional sequencing of saliva samples collected using the predicate Oragene Dx OGD-500.001 collection device (k141410). All genotyping using the GHR were performed at one site. Samples were accepted if the sample call rate (SCR) was ≥ 98%. For all samples that passed the sample call rate quality control (198/209), the genotypes were 100% (69/69 homozygous common, 65/65 heterozygous, and 64/64 homozygous rare) concordant with those from the same donor saliva samples collected in the predicate collection device and tested using bi-directional sequencing (see table below). The 95% confidence interval for all genotypes was 98.2–100% and the "no-call" rate was 0% with a 95% confidence interval of 0-1.8%. See k192944 for additional information.

Genotype	Candidate device/ Predicate device	Percent Agreement	95% Confidence Interval
GG	69/69	100%	94.8–100%
GA	65/65	100%	94.5–100%
AA	64/64	100%	94.4–100%
All genotypes	198/198	100%	98.2–100%

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):

   Performance of the AncestryDNA Saliva Collection Kit when used in the over-the-counter intended use setting of the AncestryDNA Factor V Leiden Genetic Health Risk (GHR) Test (see k192944) was evaluated. Study participants were recruited to four facilities across the U.S., representing the four U.S. census regions (i.e., South, Midwest, Northeast, and West). Participants aged 18 years of age and older were recruited to match the demographics (i.e., education attainment, age, sex at birth, and race/ethnicity) of the adult U.S. population per the most recent estimates released by the U.S. Census Bureau. Participants were asked to participate in a self-administered saliva sample collection process and completed kits were collected by the interviewer and mailed to the Ancestry Genomics laboratory for processing.

Upon receipt at the testing laboratory, each study sample was assessed for viability. For a submitted sample to be considered a success (or viable), the sample was required to pass the following three test points: (1) AncestryDNA SCK Kit Received/Accessioning (e.g., AncestryDNA SCK appropriately capped and not leaking), (2) Extraction (i.e., saliva sample is able to be extracted), and (3) DNA Call Rate (i.e., sample contains an adequate amount of DNA for testing if the sample call rate is ≥ 98%). User comprehension of test instructions, including comprehension of sample collection instructions was also assessed.

A total of 271 individuals completed the user comprehension survey and provided a saliva sample for analysis. Of the 271 samples evaluated, 14 failed one of the three test points (4 accessioning failures, no extraction failures, and 10 DNA call rate failures), resulting in a total of 257 samples passing QC metrics (94.8%). The subgroup analyses, by education attainment, age, sex at birth, and race/ethnicity, are shown below. The results demonstrated that users were able to follow sample collection instructions to obtain adequate sample for testing. See the AncestryDNA Factor V Leiden GHR Test (k192944) for additional user comprehension information.

A Flesch-Kincaid reading analysis was performed on the collection device labeling and a reading grade level of 6.0 was obtained for the overall instruction card and a reading grade level of 7.1 was obtained for the text alone.

Saliva sample evaluation – Subject Age

Age (years)	Number of participants	Accessioning failure	Sample call rate failure	Overall failures	Overall successes
Under 39	92	0/92 (0.0%)	2/92 (2.2%)	2/92 (2.2%)	90/92 (97.8%)
40 – 59	96	1/96 (1.0%)	3/95 (3.2%)	4/96 (4.2%)	92/96 (95.8%)
60+	80	3/80 (3.8%)	5/77 (6.5%)	8/80 (10.0%)	72/80 (90.0%)

Saliva sample evaluation – Subject sex at birth

Age (years)	Number of participants	Accessioning failure	Sample call rate failure	Overall failures	Overall successes
Male	136	0/136 (0.0%)	4/136 (2.9%)	4/136 (2.9%)	132/136 (97.1%)
Female	134	4/134 (3.0%)	6/130 (4.6%)	10/134 (7.5%)	124/134 (92.5%)

Saliva sample evaluation – Subject race/ethnicity

Age (years)	Number of participants	Accessioning failure	Sample call rate failure	Overall failures	Overall successes
White	187	4/187 (2.1%)	5/183 (2.7%)	9/187 (4.8%)	178/187 (95.2%)
Non-White or Multi-Race/Ethnicity	83	0/83 (0.0%)	5/83 (6.0%)	5/83 (6.0%)	78/83 (94.0%)

Saliva sample evaluation – Subject educational attainment

Age (years)	Number of participants	Accessioning failure	Sample call rate failure	Overall failures	Overall successes
High School Graduate or Less	73	0/73 (0.0%)	2/73 (2.7%)	2/73 (2.7%)	71/73 (97.3%)
Some College, including Associates Degree	104	2/104 (1.9%)	5/102 (4.9%)	7/104 (6.7%)	97/104 (93.3%)
Bachelor's Degree or Higher	93	2/93 (2.2%)	3/91 (3.3%)	5/93 (5.4%)	88/93 (94.6%)

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.