August 13, 2020

Ancestry Genomics, Inc.
Raajdeep Venkatesan
Vice President of Regulatory Affairs & Quality Assurance
153 Townsend Street, Suite 800
San Francisco, CA 94107

Re: K192947
Trade/Device Name: AncestryDNA Saliva Collection Kit
Regulation Number: 21 CFR 862.1675
Regulation Name: Blood specimen collection device
Regulatory Class: Class II
Product Code: OYJ
Dated: July 17, 2020
Received: July 20, 2020

Dear Raajdeep Venkatesan:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part

801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Marianela Perez-Torres, Ph.D.
Acting Deputy Director
Division of Chemistry
and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K192947

Device Name
AncestryDNA Saliva Collection Kit

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☐ Prescription Use (Part 21 CFR 801 Subpart D) ☒ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

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510(K) SUMMARY

A. GENERAL INFORMATION

Date Prepared: August 7, 2020

Submitter Information:

Submitted By: Ancestry Genomics, Inc.
153 Townsend Street, Suite 800
San Francisco, CA 94107

Contact Person: Raajdeep Venkatesan, MS, RAC, CMQ-OE, CBA, CQE
Vice President of RA/QA
Ancestry Genomics, Inc.
Phone: (415) 795-6110

Alternate Contact Person: Julie Wood
Director, Quality
Ancestry Genomics, Inc.
Phone: (415) 795-6000

B. PURPOSE FOR SUBMISSION

To obtain a substantial equivalence determination for the AncestryDNA Saliva Collection Kit for use in the noninvasive collection of saliva samples. DNA from the saliva sample is isolated, stabilized and suitable for over-the-counter use with the AncestryDNA Factor V Leiden Genetic Health Risk Test.

C. MEASURAND

Not applicable.

D. TYPE OF TEST

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

E. APPLICANT

Ancestry Genomics, Inc.

F. PROPRIETARY AND ESTABLISHED NAMES

AncestryDNA Saliva Collection Kit

G. REGULATORY INFORMATION

Trade Name:	AncestryDNA Saliva Collection Kit
Classification:	Class II
Regulation Number	21 CFR 862.1675
Device Classification Name:	DNA Specimen Collection, Saliva
Product Code:	OYJ
Panel:	Clinical Chemistry

H. INTENDED USE

1. Intended Use:

   See Indications for Use below.

2. Indications 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.

3. Special Conditions for Use:

   For over-the-counter use.

   AncestryDNA Saliva Collection Kit is for use with AncestryDNA Factor V Leiden Genetic Health Risk Test.

   AncestryDNA Saliva Collection Kit is only for use in adults 18 years of age and older.

4. Special Instrument Requirements:

   None.

I. DEVICE DESCRIPTION

The AncestryDNA Saliva Collection Kit consists of: saliva collection tube, funnel, cap, blister pack, collection bag with absorbent pad, return mailer, and Instructions for Use. The collection device consists of the saliva collection tube, funnel, and cap. The cap contains DNA stabilization solution. Saliva is delivered directly by spitting into the collection tube via the funnel. Once the user has provided the saliva sample, s/he removes the funnel from the saliva collection tube and affixes the cap. Affixing the cap by screwing on releases the

stabilization solution. The user is then instructed to shake the tube for at least five seconds to mix the saliva sample with the stabilization solution. After collecting the saliva sample, the user places the closed saliva collection tube in the collection bag. The collection bag with the enclosed saliva collection tube is shipped to a designated Ancestry Genomics location for testing via the pre-addressed postage paid return mailer.

J. SUBSTANTIAL EQUIVALENCE INFORMATION

1. Predicate device name(s): Oragene Dx OGD-500.001

2. Predicate 510(k) number(s): K141410

3. Comparison with predicate:

	AncestryDNA Saliva Collection Kit	**Oragene Dx OGD-500.001 (Predicate Device)**
K Number	K192947	K141410
SIMILARITIES		
Intended Use	The AncestryDNA Saliva Collection Kit is intended for use in the non-invasive collection of saliva samples. Human DNA from the saliva sample is isolated, stabilized and suitable for use with over-the-counter AncestryDNA Genetic Health Risk Tests. Saliva samples collected using the AncestryDNA Saliva Collection Kit are stabilized and can be transported and/or stored long term at ambient conditions.	Oragene®•Dx OGD-500.001 is intended for use in the non-invasive collection of saliva samples. DNA from the saliva sample is isolated, stabilized, and suitable for over-the counter use with FDA cleared, approved, or legally marketed exempt DNA carrier screening genotyping tests. Saliva samples collected using Oragene•Dx OGD-500.001 are stabilized and can be transported and/or stored long term at ambient conditions.
Special conditions for use	Over the counter	Same
Classification	Class II	Same
Analyte	DNA	Same
Sample Collection	Non-invasive collection of biological samples delivered into a non-sterile plastic collection tube	Same
Tube Material	Plastic	Same
Sample Source	Human saliva	Same
Additive	Nucleic acid stabilization solution	Same

	AncestryDNA Saliva Collection Kit	Oragene Dx OGD-500.001 (Predicate Device)
Transport and Stability	Pre-collection AncestryDNA Saliva Collection Kits can be transported at temperatures ranging from –29°C to 50°C and up to 85% RH. Post-collection AncestryDNA Saliva Collection Kits can be transported at temperatures ranging from –29°C to 50°C and up to 85% RH Pre-collection AncestryDNA Saliva Collection Kits can be stored at room temperature for up to 12 months Post-collection AncestryDNA Saliva Collection samples can be stored at room temperature for up to 12 months	Pre-collection Oragene·Dx kits can be transported at temperatures ranging from -20°C to 50°C Post-collection Oragene·Dx kits can be transported at temperatures ranging from -20°C to 50°C Pre-collection Oragene·Dx kits can be stored at room temperature for up to 24 months Post-collection Oragene·Dx samples can be stored at room temperature for up to 12 months
DIFFERENCES		
Special Conditions for Use	AncestryDNA Saliva Collection Kit is for use with AncestryDNA Factor V Leiden Genetic Health Risk Test. For use in adults 18 years and older.	For over-the-counter use. For use in adults of reproductive age.
Performance	Performance has been established with the AncestryDNA Factor V Leiden Genetic Health Risk (GHR) Test	Performance has been established with the 23andMe Personal Genome Service (PGS) Carrier Screening Test

K. 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-A1, Supplemental Tables for Interference Testing in Clinical Chemistry.

M. TEST PRINCIPLE

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. The collection device consists of the saliva collection tube, funnel, and cap. The cap contains DNA stabilization solution. Saliva is delivered directly by expectorating into the collection tube via the funnel. Once the user has provided the saliva sample, s/he 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.

N. PERFORMANCE CHARACTERISTICS

The analytical and clinical studies conducted to support the intended use and substantial equivalence claim to the predicate device are summarized below. Execution of the analytical and clinical studies and genotyping using the AncestryDNA Factor V Leiden Genetic Health Risk Test was performed by a CLIA-certified laboratory on the Illumina Infinium array platform. Results were analyzed using the Illumina iScan System and Genome Studio software to generate genotypes and to calculate call rates. Ancestry Genomics performed quality control of genotype results and associated the genotype variants to donor identification.

1. Analytical Performance

a. Reproducibility/Precision

The purpose of this study was to determine the precision and reproducibility of the AncestryDNA Factor V Leiden GHR Test at multiple sites, on multiple days, using multiple operator teams, with samples collected using multiple lots of the AncestryDNA SCK. Execution of the study protocol and genotyping using the AncestryDNA Factor V Leiden GHR Test was performed at two CLIA-certified laboratories (Lab 1 and Lab 2) on the Illumina Infinium array platform by six different operator teams (3 per laboratory).

Saliva samples were collected from nine donors with known Factor V Leiden genotypes as determined using bi-directional sequencing: three donors each with homozygous common, heterozygous, and homozygous rare. Each of the nine donors provided 19 saliva samples into three lots of AncestryDNA SCKs. This study was performed over multiple days for the AncestryDNA Factor V Leiden GHR Test evaluated in Lab 1 and Lab 2. Genotyping with the AncestryDNA Factor V Leiden GHR Test was conducted over a minimum of six non-consecutive starting days at Lab 1 and two non-consecutive days at Lab 2.

Each of the donor collections within a given AncestryDNA SCK lot were pooled and mixed, then returned to the AncestryDNA SCK tubes for double extraction. Replicates

that did not pass SCR QC in the first genotyping run underwent second, and when eligible, third genotyping run.

Single Site Precision and Repeatability

At the Lab 1 testing site, repeatability (within-run) and intermediate precision (within laboratory, across days, operator teams, and lots) was performed. Each of three operator teams tested each of the nine donor DNA samples in singlicate 20 times over five non-consecutive dates. In addition, within-run repeatability was conducted at the Lab 1 testing site. Each donor DNA sample was genotyped an additional four times on one plate (batch). Testing for this batch was performed by one operator team within a single day.

At the Lab 2 testing site, intermediate precision (within laboratory, across days, and operator teams) was performed. Each of three operator teams tested each of the nine donor DNA samples in singlicate 20 times over five non-consecutive dates.

Inter-laboratory Reproducibility

For the inter-laboratory reproducibility study, two AncestryDNA SCK Lot A saliva tubes from each donor were extracted at Lab 1 and plated to DNA plates for processing. DNA samples were tested in triplicate at Lab 1, and tested in triplicate at Lab 2. This resulted in 18 additional genotyping events per specimen (9 total replicates at Lab 1 and 9 total replicates at Lab 2). Each donor's saliva specimen was genotyped in singlet 260 times: 191 genotyping events at the Lab 1 laboratory and 69 genotyping events at the Lab 2.

The final genotyping results for the Labs 1 and 2 are summarized in the tables below. For Lab 1, testing was conducted by three different operator teams as well as saliva collection in three different AncestryDNA SCK lot combinations. For the Lab 2, testing was conducted across three different operator teams, and three different AncestryDNA SCK lot combinations. The number of concordant calls includes replicates that pass call rate QC and have genotypes concordant with the expected genotype as determined by bi-directional sequencing.

Summary of Lab 1 Testing Results

Donor ID	Expected Genotype	Total Number of Replicates	Number of Concordant Calls	Number of "No-Calls"	Number of Call Rate QC Failures	Proportion of FQC (%)
1	GG	191	191	0	0	0.00
2	GA	191	191	0	0	0.00
3	AA	191	191	0	0	0.00
4	GG	191	191	0	0	0.00
5	AA	191	191	0	0	0.00
6	GA	191	191	0	0	0.00
7	AA	191	191	0	0	0.00
8	GG	191	191	0	0	0.00
9	GA	191	188	0	3	1.57
Total		**1,719**	**1,716**	**0**	**3**	**0.17**

Summary of the Lab 2 Testing Results

Donor ID	Expected Genotype	Total Number of Replicates	Number of Concordant Calls	Number of "No-Calls"	Number of Call Rate QC Failures	Proportion of FQC (%)
1	GG	69	69	0	0	0.00
2	GA	69	69	0	0	0.00
3	AA	69	69	0	0	0.00
4	GG	69	69	0	0	0.00
5	AA	69	69	0	0	0.00
6	GA	69	69	0	0	0.00
7	AA	69	69	0	0	0.00
8	GG	69	69	0	0	0.00
9	GA	69	69	0	0	0.00
Total		**621**	**621**	**0**	**0**	**0.00**

Single Site Precision and Repeatability

Results by Site and Operator Team

The final genotyping results by site per operator team per genotype, across three AncestryDNA SCK lots is in the table below. The number of concordant calls includes replicates that pass call rate QC and have genotypes concordant with the expected genotype as determined by bi-directional sequencing.

Site and Operator Combination Results

Site/ Operator Team	Expected Genotype	Total Number of Replicates	Number of Concordant Calls	Number of "No-Calls"	Number of Call Rate QC Failures*	Proportion of FQC (%)
Lab 1 Team 1	**AA**	195	195	0	0	0.00
	GA	195	194	0	1	0.51
	GG	195	195	0	0	0.00
Total		***585***	***584***	***0***	***1***	***0.17***
Lab 1 Team 2	**AA**	189	189	0	0	0.00
	GA	189	188	0	1	0.53
	GG	189	189	0	0	0.00
Total		***567***	***566***	***0***	***1***	***0.18***
Lab 1 Team 3	**AA**	189	189	0	0	0.00
	GA	189	188	0	1	0.53
	GG	189	189	0	0	0.00
Total		***567***	***566***	***0***	***1***	***0.18***
Lab 2 Team 1	**AA**	69	69	0	0	0.00
	GA	69	69	0	0	0.00
	GG	69	69	0	0	0.00
Total		***207***	***207***	***0***	***0***	***0.00***
Lab 2	**AA**	69	69	0	0	0.00

Site/ Operator Team	Expected Genotype	Total Number of Replicates	Number of Concordant Calls	Number of "No-Calls"	Number of Call Rate QC Failures*	Proportion of FQC (%)
Team 2	**GA**	69	69	0	0	0.00
	GG	69	69	0	0	0.00
Total		***207***	***207***	***0***	***0***	***0.00***
Lab 2 Team 3	**AA**	69	69	0	0	0.00
	GA	69	69	0	0	0.00
	GG	69	69	0	0	0.00
Total		***207***	***207***	***0***	***0***	***0.00***
All teams' totals combined	**GG, GA, AA**	**2,340**	**2,337**	**0**	**3**	**0.13**

* The heterozygous donor failures in each of the Lab 1 operator teams are from Donor 9 listed above.

Results by Site and AncestryDNA SCK Lot Combination

The final genotyping results by site per AncestryDNA SCK lot combination per genotype across six different operator teams and three AncestryDNA SCK lots are in the table below. The number of genotype calls includes replicates that pass the QC call rate and have genotypes concordance with the expected genotype as determined by bi-directional sequencing.

Site and AncestryDNA SCK Lot Combination Results

Site/AncestryDNA SCK Lot	Expected Genotype	Total Number of Replicates	Number of Concordant Calls	Number of "No-Calls"	Number of Call Rate QC Failures	Proportion of FQC (%)
Lab 1-Lot A	AA	213	213	0	0	0.00
	GA	213	213	0	0	0.00
	GG	213	213	0	0	0.00
Total		***639***	***639***	***0***	***0***	***0.00***
Lab 1-Lot B	AA	180	180	0	0	0.00
	GA	180	177	0	3	1.67
	GG	180	180	0	0	0.00
Total		***540***	***537***	***0***	***3***	***0.56***
Lab 1-Lot C	AA	180	180	0	0	0.00
	GA	180	180	0	0	0.00
	GG	180	180	0	0	0.00
Total		***540***	***540***	***0***	***0***	***0.00***
Lab 2-Lot A	AA	87	87	0	0	0.00
	GA	87	87	0	0	0.00
	GG	87	87	0	0	0.00
Total		***261***	***261***	***0***	***0***	***0.00***

Site/AncestryDNA SCK Lot	Expected Genotype	Total Number of Replicates	Number of Concordant Calls	Number of "No-Calls"	Number of Call Rate QC Failures	Proportion of FQC (%)
Lab 2-Lot B	AA	60	60	0	0	0.00
	GA	60	60	0	0	0.00
	GG	60	60	0	0	0.00
Total		***180***	***180***	***0***	***0***	***0.00***
Lab 2-Lot C	AA	60	60	0	0	0.00
	GA	60	60	0	0	0.00
	GG	60	60	0	0	0.00
Total		***180***	***180***	***0***	***0***	***0.00***
All AncestryDNA SCK lots' totals combined	**GG, GA, AA**	**2,340**	**2,337**	**0**	**3**	**0.13**

Within-Run Repeatability

Within-run repeatability was conducted at the Lab 1 testing site and the results are in the table below. Each donor DNA sample was genotyped four additional times on one plate (batch). There were no genotyping repeats.

Within-Run Repeatability Results from Lab 1

Donor ID	Expected Genotype	Total Number of Replicates	Number of Concordant Calls	Number of "No-Calls"	Number of Call Rate QC Failures	Proportion of FQC (%)
1	GG	5	5	0	0	0.00
2	GA	5	5	0	0	0.00
3	AA	5	5	0	0	0.00
4	GG	5	5	0	0	0.00
5	AA	5	5	0	0	0.00
6	GA	5	5	0	0	0.00
7	AA	5	5	0	0	0.00
8	GG	5	5	0	0	0.00
9	GA	5	5	0	0	0.00
Totals		**45**	**45**	**0**	**0**	**0.00**

Inter-laboratory Reproducibility

The final genotyping results for the inter-laboratory reproducibility study (n=2) across six operator teams is in the table below. The number of concordant calls includes replicates that pass call rate quality control and also have genotypes concordant with the expected genotype (bi-directional sequencing genotype result).

Inter-Laboratory Reproducibility

Donor ID	Expected Genotype	Total Number of Replicates (Lab 1| *Lab 2*)		Number of Concordant Calls (Lab 1| *Lab 2*)		Number of "No-Calls" (Lab 1| *Lab 2*)		Number of Call Rate QC Failures (Lab 1| *Lab 2*)		Proportion of FQC (%) (Lab 1| *Lab 2*)	
1	GG	9	9	9	9	0	0	0	0	0.00	0.00
2	GA	9	9	9	9	0	0	0	0	0.00	0.00
3	AA	9	9	9	9	0	0	0	0	0.00	0.00
4	GG	9	9	9	9	0	0	0	0	0.00	0.00
5	AA	9	9	9	9	0	0	0	0	0.00	0.00
6	GA	9	9	9	9	0	0	0	0	0.00	0.00
7	AA	9	9	9	9	0	0	0	0	0.00	0.00
8	GG	9	9	9	9	0	0	0	0	0.00	0.00
9	GA	9	9	9	9	0	0	0	0	0.00	0.00
Totals		**81**	**81**	**81**	**81**	**0**	**0**	**0**	**0**	**0.00**	**0.00**

Overall Percent Agreement (OPA) for Repeatability and Genotyping

The OPA point estimates for repeatability exceeded the 99% predefined protocol acceptance criteria, and the OPA point estimates for each genotype exceeded the 99% predefined protocol acceptance criteria as seen in the table below.

Point Estimates for Overall Percent Agreement for Repeatability and Genotype

Attribute	Concordant Replicates (Total QC Passing Replicates)	Point Estimate Percent Agreement (%) (95% Confidence Interval)
Lab 1 laboratory	1,716 (1,716)	100.00 (99.79 – 100.00)
Lab 2 laboratory	621 (621)	100.00 (99.41 – 100.00)
Lab 1 operator team 1	584 (584)	100.00 (99.37 – 100.00)
Lab 1 operator team 2	566 (566)	100.00 (99.35 – 100.00)
Lab 1 operator team 3	566 (566)	100.00 (99.35– 100.00)
Lab 2 operator team 1	207 (207)	100.00 (98.23 – 100.00)
Lab 2 operator team 2	207 (207)	100.00 (98.23 – 100.00)
Lab 2 operator team 3	207 (207)	100.00 (98.23 – 100.00)
All operator teams	2,337 (2,337)	100.00 (99.84 – 100.00)
Lab 1 AncestryDNA SCK lot A	639 (639)	100.00 (99.42 – 100.00)
Lab 1 AncestryDNA SCK lot B	537 (537)	100.00 (99.32 – 100.00)
Lab 1 AncestryDNA SCK lot C	540 (540)	100.00 (99.32 – 100.00)
Lab 2 AncestryDNA SCK lot A	261 (261)	100.00 (98.60– 100.00)
Lab 2 AncestryDNA SCK lot B	180 (180)	100.00 (97.97 – 100.00)
Lab 2 AncestryDNA SCK lot C	180 (180)	100.00 (97.97 – 100.00)
All AncestryDNA SCK lot combinations	2,337 (2,337)	100.00 (99.84 – 100.00)
Within-run repeatability	45 (45)	100.00 (92.13 – 100.00)
Inter-lab data at Lab 1	81 (81)	100.00 (95.55 – 100.00)
Inter-lab data at Lab 2	81 (81)	100 .00 (95.55 – 100.00)
All "GG"	780 (780)	100.00 (99.53 – 100.00)
All "GA"	777 (777)	100.00 (99.53 – 100.00)

Attribute	Concordant Replicates (Total QC Passing Replicates)	Point Estimate Percent Agreement (%) (95% Confidence Interval)
All "AA"	780 (780)	100.00 (99.53 – 100.00)

The AncestryDNA Factor V Leiden GHR Test was evaluated at multiple labs, on multiple days, by multiple personnel teams. The results demonstrate that the AncestryDNA Factor V Leiden GHR Test met the acceptance criteria for overall precision ≥ 99% point estimate, and for each genotype ≥ 99% agreement. The AncestryDNA Factor V Leiden GHR Test also delivered precise and reproducible results across multiple AncestryDNA SCK lots. The AncestryDNA SCK in combination with the AncestryDNA Factor V Leiden GHR Test consistently produced results that were in agreement with the true variant status, which was determined by bidirectional sequencing.

b. Linearity/Assay Reportable Range

Not applicable.

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

Pre-collection AncestryDNA Saliva Collection Kits can be stored at 15°C to 30°C for up to 12 months.

Pre-collection AncestryDNA Saliva Collection Kits can be transported at temperatures ranging from –29°C to 50°C and up to 85% RH.

d. Analytical Sensitivity

The study was designed around the regression (probit/logit) approach from section 5.5 of the CLSI EP17-A2 to determine Limit of Blank (LoB) and Limit of Detection (LoD). Each sample was serially diluted to different DNA concentrations and genotyped. To confirm the genotype call, each sample was also sequenced by bi-directional sequencing to determine the rates of correct genotype calls at each DNA concentration. The Limit of Detection (LoD) was defined as the lowest DNA concentration at which at least 95% of samples yielded the correct call.

Limit of Blank Test Method
Due to the noisy nature of UV-Vis spectroscopy and potentially erroneous fluorescence signal in the assay, to establish the Limit of Blank (LoB) samples were extracted and quantified on a plate of 95 blanks containing 1 mL of molecular grade water and the standard volume of DNA stabilizing solution from the AncestryDNA SCK. The samples were extracted, quantified, and genotyped.

Limit of Detection Test Method

Saliva samples were collected from 15 donors with known Factor V Leiden genotypes as determined using bi-directional sequencing: five (5) donors each with homozygous common, heterozygous, and homozygous rare. Samples were collected using the Oragene® Dx Collection Device, model OGD-500.001 (OGD) (n=1) and the AncestryDNA SCK (n=4). DNA extracted from each donor's saliva samples were pooled to create a homogenous solution. The pooled DNA was used to create a two-fold dilution series, including a neat sample and four additional dilutions for a total of five samples per donor per genotype in the series. Six replicates of each dilution series were genotyped using the AncestryDNA Factor V Leiden GHR Test. Testing was performed under standard protocol for the AncestryDNA GHR Test, except that each donor sample in the series was genotyped in duplicate. A total 450 replicates were tested in the LoD study.

The LoB = 1.004 ng/µL, based on the non-parametric rank method from section 5.3.3.1 of EP17-A2 to account for sources of measurement variability in the both UV-Vis spectrophotometry and the bead-based fluorescence assay.

The LoD = 1.53 ng/µL, a limit concentration that is statistically distinguishable from blank samples.

The upper limit of concentration = 50 ng/µL.

All genotyping attempts on samples containing the measurand with call rates ≥ 98% and concentrations between 1.53 ng/µL and 50 ng/µL produced genotypes concordant with bidirectional sequencing.

e. Interfering Substances

The analytical specificity studies were designed using *CLSI EP07 – Interference Testing in Clinical Chemistry*; Approved Guideline – Third Edition for determining potential interference with the AncestryDNA Factor V Leiden GHR Test. Endogenous, exogenous, and microbial DNA were evaluated as part of the analytical specificity study.

Endogenous Interference

Four potential common endogenous interferents were evaluated to determine the effect on the performance of the AncestryDNA Factor V Leiden GHR Test as listed in the table below.

Endogenous Interferent Concentrations

Endogenous Substance	Final Concentration (1x) in Saliva
PBS (reference/control)	N/A
Salivary α-amylase	395 U/mL
Hemoglobin	20 mg/mL
IgA	0.44 mg/mL
Total Protein	0.185 mg/mL Salivary α-amylase
	0.44 mg/mL IgA

Endogenous Substance	Final Concentration (1x) in Saliva
	2.05 mg/mL human serum albumin

A total of ten total saliva donors with unknown Factor V Leiden genotypes were utilized in the specificity study. A saliva sample from each donor was collected with the Oragene® Dx Collection Device, model OGD-500.001 (OGD) (K141410) and sent to a third party laboratory to determine the true variant status using bi-directional sequencing analysis. Each donor provided five saliva samples into five AncestryDNA SCK Saliva Collection Tubes that were shipped to the laboratory. The endogenous substances were individually spiked into saliva prior to DNA extraction and genotyping. Saliva that was spiked with PBS served as the reference/control. The assay was executed by the same two (2) operators for each genotyping replicate. Each sample was genotyped in triplicate for a total of 30 replicate genotyping attempts (3 replicates for each of 10 donors) per each interferent and 30 control replicate genotyping attempts (450 total initial genotyping attempts).

For all samples (control samples and samples with endogenous interferents) that passed quality control, the concordance was 100%, exceeding the acceptance criterion of ≥ 95% agreement with true variant status as determined by bi-directional sequencing. The point estimate of overall percent agreement from each of the endogenous interferents is provided in the table below. The results indicate that the performance of the AncestryDNA Factor V Leiden GHR Test when tested from samples collected with the AncestryDNA SCK are not affected by the tested interferents.

Overall Percent Agreement for the Endogenous Interference Study

Endogenous Interferent	Overall Percent Agreement Point Estimate
PBS (reference/control)	100% (30/30)
Salivary α-amylase	100% (30/30)
Hemoglobin	100% (30/30)
IgA	100% (30/30)
Total Protein	100% (30/30)

Exogenous Interference

Six potential exogenous interferents were evaluated to determine its effect on the performance of the AncestryDNA Factor V Leiden GHR Test. The exogenous interference study included samples from non-smokers and smokers. Saliva samples were collected from 10 non-smokers in 15 AncestryDNA SCKs over the course of five (5) days. Each day, the donor performed one (1) of the five (5) activities (eating beef, eating chicken, drinking alcohol, chewing gum, or using mouthwash). The donors provided three (3) tubes per day as follows: before consuming the exogenous substance (control/baseline), immediately after, and 30-minutes after performing the activity. Saliva samples were also collected from 10 smokers into 3 AncestryDNA SCK per day as follows: before smoking (control/baseline), immediately after smoking, and 30-minutes after smoking. There was a total of 594 data points as summarized in the table below.

Overview of Exogenous Interferent Study Design

Exogenous Activity	Donor Count	Time Point	Replicates	Total
Eating 3 oz. chicken	12	3	3	108
Drinking 1.5 oz. alcohol	12	3	3	108
Using mouthwash	12	3	3	108
Eating 3 oz beef	10	3	3	90
Chewing gum	10	3	3	90
Smoking	10	3	3	90
Total				**594**

For all samples where the control samples and replicates containing the interfering substances passed QC, the concordance for all interfering substances was 100% met the acceptance criterion of ≥ 95% agreement with true variant status from bi-directional sequencing for all samples that have passed QC. Results indicate that the performance of the AncestryDNA Factor V Leiden GHR Test when tested from samples collected with the AncestryDNA SCK are not affected by the tested interferents. The table below **summarizes** the overall percent agreement (OPA) point estimate calculated on genotyping events with control samples that passed QC for each interferent and time point.

Overall Percent Agreement for Exogenous Interferents

Exogenous Substance	OPA Point Estimate (Concordant Replicates / Total QC Passing Replicates)	
	T0	T30
Chicken	100% (33/33)	100% (36/36)
Alcohol	100% (36/36)	100% (36/36)
Mouthwash	100% (36/36)	100% (36/36)
Beef	100% (27/27)	100% (30/30)
Gum	100% (30/30)	100% (30/30)
Smoking	100% (30/30)	100% (30/30)

For the eating chicken and eating beef activities, a higher rate of QC failures for samples collected immediately after completing the activity (T0) when matched donor control samples passed QC was observed. Therefore, the AncestryDNA Saliva Collection Kit will include in the labeling 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.

Microbial Interference

Microbial DNA from five (5) different species (*Staphylococcus epidermis*, *Streptococcus mutans*, *Lactobacillus casei*, *Actinomyces odontolyticus*, and *Candida albicans)* were evaluated to determine its impact on the performance of the AncestryDNA Factor V Leiden GHR Test. DNA from six (6) human cell lines was obtained for this study:

- Four cell lines were Factor V Leiden homozygous common (wild type; GG),
- One cell line was Factor V Leiden heterozygous (GA), and
- One cell line was Factor V Leiden homozygous rare (Variant; AA).

All cell lines were subjected to bi-directional sequencing by a third-party laboratory to verify the Factor V Leiden genotype as part of the study. DNA from each of the six human cell lines was spiked with two concentrations (low/normal (2.8 ng/µL) and high (12.5 ng/µL)) of the five different species of microbial DNA. Human cell line DNA spiked with buffer functioned as a spike-in control at both concentrations. Each of the human cell lines was spiked a total of 12 times (5 microbial interferents and a control at 2 levels per cell line). The resulting 72 DNA mixtures were genotyped in replicates of 6 using the AncestryDNA Factor V Leiden GHR Test, for a total of 432 genotyping results (6 cell lines x 6 microbe/control x 2 concentrations x 6 replicates = 432).

Each sample and replicate, spiked with two levels of microbial interferent, and unspiked (spiked with PBS) was compared directly to bidirectional sequencing results. The assay produced concordant genotypes with bidirectional sequencing in all genotyping events. The point estimate of overall percent agreement (OPA) with true variant status from each condition is provided below. The Factor V Leiden GHR Test performs to the internal specifications and meets the study acceptance criteria of a ≥ 95% agreement with true variant status. The assay reproduced the true variant status, as determined by bidirectional sequencing, for each replicate that was tested, including all control genotyping replicates and all interferent-spiked genotyping replicates. The results indicate that there is no significant impact of common microbial interferents on the performance of the Factor V Leiden GHR Test in either low/normal or higher-than-average concentrations.

Microbial Interferent Testing Results for Overall Percent Agreement

Microbial Interferent	OPA Point Estimate	
	Low/Normal Concentration	High Concentration
Buffer (reference/control)	100% (36/36)	100% (36/36)
S. epidermis	100% (36/36)	100% (36/36)
S. mutans	100% (36/36)	100% (36/36)
L. casei	100% (36/36)	100% (36/36)
A. odontolyticus	100% (36/36)	100% (36/36)
C. albicans	100% (36/36)	100% (36/36)

f. Assay Cut-off

Not applicable.

g. Sample Volume Tolerance

Saliva sample volume in the AncestryDNA SCK was evaluated to determine its effect on the performance of the AncestryDNA Factor V Leiden GHR Test. Saliva samples were collected from 80 donors with known Factor V Leiden genotypes: 60 homozygous common (wild type), 15 heterozygous, and five homozygous rare (variant). Each of the 80 donors provided saliva samples into four tubes for a total of n=320 tubes for testing with the AncestryDNA Factor V Leiden GHR Test (240 tubes) and bi-directional sequencing analysis (80 tubes). For each donor, one AncestryDNA SCK Saliva

Collection Tube was under-filled, one was filled per the instructions for use, and one was over-filled. All of the AncestryDNA SCK Saliva Collection Tubes were genotyped with the AncestryDNA Factor V Leiden GHR Test.

Nine out of 240 samples failed sample call rate QC. The AncestryDNA Factor V Leiden GHR Test reproduced the true variant status for all samples that passed QC irrespective of fill volume as determined by bi-directional sequencing. For all target fill volumes evaluated, the acceptance criterion was met which stated, for all samples that passed quality control for each collection volume (under-filled, normally filled, over-filled), ≥ 95% agreement with true variant status. The point estimates of overall percent agreement (OPA) with the bidirectional sequencing and AncestryDNA Factor V Leiden GHR Test showed 100% agreement across all fills and genotypes evaluated in the tables below.

Overall Percent Agreement Point Estimates Stratified by Fill Line

Fill	Fill Line	Concordant QC Passing Samples / Total QC Passing Samples	Point Estimate OPA (%)
Control	**1.0 mL**	79/79	100
Under	**0.5 mL**	79/79	100
Over	**1.5 mL**	73/73	100

Overall Percent Agreement Point Estimates Stratified by Genotype

Genotype by Sequencing		Concordant QC Passing Samples / Total QC Passing Samples	Point Estimate OPA (%)
Wild Type	GG	172/172	100
Heterozygous	GA	44/44	100
Variant	AA	15/15	100

h. Specimen Stability

Post-collection AncestryDNA Saliva Collection samples can be stored at 15°C to 30°C for up to 12 months.

Post-collection AncestryDNA Saliva Collection Kits can be transported at temperatures ranging from –29°C to 50°C and up to 85% RH.

2. Comparison Studies

a. Method Comparison with the Predicate

The accuracy of the AncestryDNA Factor V Leiden GHR Test was established by comparing the results of the test to the true variant status as determined by bi-directional sequencing analysis at a third-party laboratory.

Saliva samples were collected from 209 donors with known Factor V Leiden genotypes: 200 initial study donors plus nine alternate study donors. The genotypes of the donors as determined by bi-directional sequencing were 73 homozygous common, 69

heterozygous, and 67 homozygous rare. Samples were collected using Oragene Dx Ogd-500.001 (OGD) and the AncestryDNA SCK.

- Each sample collected using the OGD device was subjected to bi-directional sequencing by a third-party laboratory to verify the Factor V Leiden genotype,
- Each donor sample collected using the AncestryDNA SCK was used in the accuracy study with the AncestryDNA Factor V Leiden GHR Test.

Of the 200 samples initially genotyped, 185 passed the first pass genotyping quality control sample-level call rate (SCR) of ≥ 98% and 15 did not (<98%). Nine alternate samples were added to the sample cohort, all of which passed the first pass genotyping SCR of ≥ 98%. The 15 samples that were regenotyped, four passed SCR quality control in second pass genotyping attempts, and 11 failed SCR quality control in the second pass genotyping attempt and were not eligible for a third genotyping attempt based on sample call rate criteria. The table below summarizes the genotype counts for all samples passing SCR quality control for the AncestryDNA Factor V Leiden GHR Test and the genotyping results for the bi-directional sequencing. Zero (0) 'no-call' events were observed in any of the samples that passed quality control.

Comparison of the Genotyping Results for Bi-directional Sequencing and AncestryDNA Factor V Leiden GHR Test

		Bi-directional Sequencing Genotypes			Total
		GG	*GA*	*AA*	
AncestryDNA Factor V Leiden GHR Test Genotypes	*GG (homozygous common)*	69	0	0	69
	GA (heterozygous)	0	65	0	65
	AA (homozygous rare)	0	0	64	64
	00 (no-call)	0	0	0	0
Total		69	65	64	198

For all samples that passed quality control, the overall percent agreement and the percent agreement for each genotype with bi-directional sequencing genotypes was 100% as outlined below.

Percent Agreement and Confidence Intervals for AncestryDNA Factor V Leiden GHR Test Genotypes as Compared with Bi-directional Sequencing

Genotype	Observed/Expected	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%

The calculated overall percent agreement and 95% confidence intervals based on a binomial distribution between genotypes obtained from donors using the AncestryDNA SCK with the AncestryDNA Factor V Leiden GHR Test and using the Oragene Dx Ogd-500.001 (OGD) for bi-directional sequencing.

Overall Percent Agreement and Confidence Interval for Genotypes Obtained Using AncestryDNA SCK Compared to OGD

Proportion Concordant Genotypes (AncestryDNA SCK vs. OGD)	Genotype OPA Between Saliva Collection Devices	95% Confidence Interval
198/198	100%	98.2 – 100%

The genotype frequencies for Factor V Leiden in various US population were obtained from the 2018 ACMG reporting standard (Zhang et al., 2018): "In the United States, factor V Leiden heterozygosity is present in 5.1%, 2.0%, and 1.2% of Caucasians, Hispanics, and African Americans respectively; the frequencies of homozygosity for the above populations are 65, 10, and 4 per 100,000 individuals correspondingly."

In the accuracy study for the AncestryDNA Factor V Leiden GHR Test, point estimates of PA(GG|GG) = PA(GA|GA)=PA(AA|AA) = 100%. The point estimate for the Technical Positive Predictive Value (TPPV) for such a scenario is 100% for both heterozygotes and homozygotes. While this point estimate may have larger uncertainty about the TPPV than an estimate from a larger test population, no discordant genotypes were observed in any of the 198 genotyping events that passed quality control.

For all samples that passed SCR quality control (198/209, 95%), the AncestryDNA Factor V Leiden GHR Test genotypes were 100% concordant with true variant status, determined by bi-directional sequencing from saliva samples collected in the OGD. This result met the predefined protocol acceptance criterion of ≥ 99% agreement with true variant determination overall and per genotype tested. These results show that the AncestryDNA SCK is capable of noninvasive collection of saliva samples for the purpose of supporting DNA tests that evaluate genomic DNA isolated from the collection device.

b. Matrix Comparison

Not applicable.

3. Clinical Studies

a. User Comprehension Study

Ancestry Genomics sponsored a study to assess naive user comprehension of labeling provided with the AncestryDNA SCK. The user comprehension study was performed using a sampling of individuals that was demographically diverse, using quota-based sampling methods and was conducted in a controlled environment at four sites across the U.S. In addition to the assessment of user comprehension of the SCK labeling, the study was moderated in order to collect observational data on participants' overall experience.

The user comprehension study was designed to assess the comprehension of representative labeling contained in the AncestryDNA Sample Collection Kit. The study was conducted with demographically diverse individuals to evaluate the reading level required for comprehension of the instructions for use.

The user comprehension study was conducted to:

a) evaluate readability of all written materials and instructions in the SCK
b) document the SCK user experience, including submitting samples to the lab for testing to determine their viability to generate a genotype

Participants were representative of the intended use population: adults aged 18 years and older in the U.S. Participants were recruited to match the demographics (education, age, sex/gender, and race/ethnicity) of the adult U.S. population as of the most recent estimates released by the U.S. Census Bureau. Geographic diversity was addressed through participant recruitment and comprehension testing from each of the four (4) U.S. Census geographic regions: Northeast, Midwest, South, and West.

351 individuals were recruited for the study using quota sampling methods. Of the 351 individuals recruited, 271 participants were enrolled in the study. They were provided with the AncestryDNA SCK, instructed to open the kit, and follow all instructions to prepare the kit exactly as they would if they were doing so at home. After the procedure, the participant responded to an online post survey and was interviewed about the experience using the AncestryDNA SCK. The samples were shipped to a central CLIA certified laboratory, processed for viability and reported any failures in accessioning, extraction, or deficiency in DNA. AncestryDNA SCKs were only processed to determine that the sample contained an adequate amount of viable DNA to pass the call rate of ≥ 98%.

Out of the 271 individuals who completed the user comprehension survey and provided a saliva sample for analysis, 257 (94.8%) of the submitted saliva collection kit samples passed through all three evaluated failure points, and passed sample call rate of 98%. See Ancestry Genomics co-submission K192944 for additional user study information.

The AncestryDNA SCK instructions for use was calculated to have a Flesch-Kincaid reading level of 6.0, and the text alone reading level result was 7.1. The success criterion, that the Flesch-Kinkaid reading level shall meet or exceed the 8th grade threshold, was met.

Participants rated the AncestryDNA SCKs instructions as easy to understand and easy to follow and rated the illustrations as helpful.

- 98.1% rated the overall usability of the test as somewhat easy or higher.
- 99.2% rated the kit instructions as somewhat easy to understand or better.
- 98.1% indicated that the instructions were somewhat easy to follow or better.
- 99.6% said the illustrations on the card were somewhat helpful or better.

b. Expected Values/Reference Range

Not applicable.

O. INSTRUMENT NAME

Not applicable.

P. PROPOSED LABELING

The labeling satisfies the requirements of 21 CFR Parts 801 and 809.

Q. CONCLUSION

The results of the analytical, clinical and user studies submitted in this 510(k) Premarket Notification are complete and demonstrate that the AncestryDNA Saliva Collection Kit meets the established specifications necessary for consistent performance for the intended use of noninvasive collection, stabilization, transportation, and storage of saliva samples. The results support a conclusion that the AncestryDNA Saliva Collection Kit is substantially equivalent to the predicate.