August 13, 2020

Ancestry Genomics, Inc.
Raaj Venkatesan
Associate Director, Regulatory Affairs
153 Townsend Street, Suite 800
San Francisco, California 94107

Re: K192944
Trade/Device Name: AncestryDNA Factor V Leiden Genetic Health Risk Test
Regulation Number: 21 CFR 866.5950
Regulation Name: Genetic Health Risk Assessment System
Regulatory Class: Class II
Product Code: PTA
Dated: October 17, 2019
Received: October 18, 2019

Dear Raaj 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,

Takeesha Taylor-Bell
Chief
Division of Immunology
and Hematology 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)*
K192944

Device Name
AncestryDNA Factor V Leiden Genetic Health Risk Test

Indications for Use *(Describe)*
The AncestryDNA Factor V Leiden Genetic Health Risk Test for Hereditary Thrombophilia uses qualitative genotyping to detect clinically relevant variants in genomic DNA isolated from human saliva collected from individuals 18 years and older with the AncestryDNA Saliva Collection Kit for the purpose of reporting and interpreting Genetic Health Risks (GHR).

The AncestryDNA Factor V Leiden Genetic Health Risk Report for Hereditary Thrombophilia is indicated for reporting of the Factor V Leiden variant in the F5 gene. This report describes if a person has variants associated with a higher risk of developing harmful blood clots, but it does not describe a person's overall risk of developing harmful blood clots. This test is most relevant for people of European descent.

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.

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510(K) SUMMARY

A. GENERAL INFORMATION

Submission Date: October 17, 2019

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.

Alternate Contact Person: Julie Wood
Director of Quality
Ancestry Genomics, Inc.

B. PURPOSE FOR SUBMISSION

To obtain a substantial equivalence determination for the AncestryDNA Factor V Leiden Genetic Health Risk Test.

C. MEASURAND

Factor V Leiden c.1601G>A variant in the F5 gene

D. TYPE OF TEST

Qualitative in vitro molecular diagnostic system

E. APPLICANT

Ancestry Genomics, Inc.

F. PROPRIETARY AND ESTABLISHED NAMES

AncestryDNA Factor V Leiden Genetic Health Risk Test

G. REGULATORY INFORMATION

Trade Name:	AncestryDNA Factor V Leiden Genetic Health Risk Test
Classification:	Class II
Regulation:	21 CFR 866.5950
Regulation Name:	Genetic health risk assessment system
Product Code:	PTA
Panel:	Immunology

H. INTENDED USE

See Indications for Use below.

I. INDICATIONS FOR USE

1. Indications for Use:

   The AncestryDNA Factor V Leiden Genetic Health Risk Test for Hereditary Thrombophilia uses qualitative genotyping to detect clinically relevant variants in genomic DNA isolated from human saliva collected from individuals 18 years and older with the AncestryDNA Saliva Collection Kit for the purpose of reporting and interpreting Genetic Health Risks (GHR).

   The AncestryDNA Factor V Leiden Genetic Health Risk Report for Hereditary Thrombophilia is indicated for reporting of the Factor V Leiden variant in the F5 gene. This report describes if a person has variants associated with a higher risk of developing harmful blood clots, but it does not describe a person's overall risk of developing harmful blood clots. This test is most relevant for people of European descent.

2. Special Conditions for Use Statements:

   a. For over-the-counter (OTC) use.
   b. This test is not a substitute for visits to a healthcare provider. It is recommended that you consult with a healthcare provider if you have any questions or concerns about your results.
   c. The AncestryDNA Factor V Leiden Genetic Health Risk (GHR) Test does not detect all genetic variants associated with Hereditary Thrombophilia. The absence of a variant tested does not rule out the presence of other genetic variants that may be disease-related.
   d. The test is intended for users ≥ 18 years old.
   e. The test does not diagnose any specific health conditions. Results should not be used to make medical decisions.
   f. The laboratory may not be able to process a user's sample. The probability that the laboratory cannot process a sample can be up to 3% (This estimate was obtained from samples processed in one lab only). If this happens, we will notify you by e-mail and you may request one free replacement kit to provide us with a new sample.

g. A user's race, ethnicity, age, and other lifestyle factors may affect how the genetic test results are interpreted.
h. Subject to meeting the limitations contained in the special controls under regulation 21 CFR 866.5950.

3. Special Instrument Requirements:

The AncestryDNA Factor V Leiden Genetic Health Risk Test is to be performed using the Tecan Evo and Illumina iScan instruments.

GenomeStudio Software is a modular software application that is used to view and analyze genotypic data obtained from the Illumina iScan System and based on the cluster definition file. AncestryDNA GHR software conducts a variety of control checks on the file, resulting in a final genotype profile for each sample. These data are used to generate test reports on a user's genotype and associated risk of disease.

J. DEVICE DESCRIPTION

A user's saliva is self-collected using the AncestryDNA Saliva Collection Kit, which consists of a sealable collection tube containing a stabilizing buffer solution. Once the sample is collected, it is shipped to one of two Clinical Laboratory Improvement Amendments (CLIA) certified laboratories for processing.

DNA is isolated from the saliva, quantified, and tested in a multiplex assay using a customized genotyping chip and instrumentation manufactured by Illumina. The multiplex assay simultaneously tests for more than 500,000 variants, including those for the indication proposed herein.

The raw data is generated using Illumina GenomeStudio software, and then sent to Ancestry Genomics (the Manufacturer). The data are analyzed using the Manufacturer's proprietary GHR software, and a genotype is determined for each tested variant. The results for the Factor V Leiden variant are used to generate personalized reports for users that provide information about the disease associated with the detected variant.

Personalized reports are generated for each user that provide results of the testing performed. These reports tell the user which variant(s) has/have been detected in their sample and provide information on the risk of disease associated with the variant(s). If no variant was detected, that information is also provided. The personalized reports are designed to present scientific concepts to users in an easy-to-understand format. The reports provide scientifically valid information about the risks associated with the presence of a particular variant. The reports are designed to help users understand the meaning of their results and any appropriate actions that may be taken based on their results.

K. SUBSTANTIAL EQUIVALENCE INFORMATION

1. Predicate device name(s):
   23andMe Personal Genome Service (PGS) Test

2. Predicate 510(k) number(s):
   DEN160026

3. Comparison with predicate:

Table 5-1: Predicate Device Comparison

	AncestryDNA Factor V Leiden Genetic Health Risk Test	23andMe Personal Genome Service Test (Predicate Device)
K Number	K192944	DEN160026/DEN140044
SIMILARITIES		
Intended Use	The AncestryDNA Factor V Leiden Genetic Health Risk Report for Hereditary Thrombophilia is indicated for reporting of the Factor V Leiden variant in the F5 gene. This report describes if a person has variants associated with a higher risk of developing harmful blood clots, but it does not describe a person's overall risk of developing harmful blood clots. This test is most relevant for people of European descent.	The 23andMe PGS Genetic Health Risk Report for Hereditary Thrombophilia is indicated for reporting of the Factor V Leiden variant in the F5 gene, and the Prothrombin G20210A variant in the F2 gene. This report describes if a person has variants associated with a higher risk of developing harmful blood clots, but it does not describe a person's overall risk of developing harmful blood clots. This test is most relevant for people of European descent.
Special Conditions for Use Statements	a. For over-the-counter (OTC) use. b. This test is not a substitute for visits to a healthcare provider. It is recommended that you consult with a healthcare provider if you have any questions or concerns about your results. c. The AncestryDNA Factor V Leiden Genetic Health Risk Test does not detect all genetic variants associated with Hereditary Thrombophilia. The absence of a variant tested does not rule out the presence of other genetic variants that may be disease-related.	a. For over-the-counter (OTC) use. b. This test is not a substitute for visits to a healthcare provider. It is recommended that you consult with a healthcare provider if you have any questions or concerns about your results. c. The 23andMe PGS Genetic Health Risk Tests for Hereditary Thrombophilia do not detect all genetic variants associated with the aforementioned diseases. The absence of a variant tested does not rule out the presence of other genetic variants that may be disease-related.

	AncestryDNA Factor V Leiden Genetic Health Risk Test	**23andMe Personal Genome Service Test (Predicate Device)**
	d. The test is intended for users ≥ 18 years old. e. The test does not diagnose any specific health conditions. Results should not be used to make medical decisions. f. The laboratory may not be able to process a user's sample. The probability that the laboratory cannot process a sample can be up to 3% (This estimate was obtained from samples processed in one lab only). If this happens, we will notify you by e-mail and you may request one free replacement kit to provide us with a new sample. g. A user's race, ethnicity, age, and other lifestyle factors may affect how the genetic test results are interpreted. h. Subject to meeting the limitations contained in the special controls under regulation 21 CFR 866.5950.	d. The test is intended for users ≥ 18 years old. e. The test does not diagnose any specific health conditions. Results should not be used to make medical decisions. f. The laboratory may not be able to process a user's sample. The probability that the laboratory cannot process a sample can be up to 7.6%. g. A user's race, ethnicity, age, and sex may affect how the genetic test results are interpreted. h. Subject to meeting the limitations contained in the special controls under regulation 21 CFR 866.5950.
Special Instrument Requirements	The AncestryDNA Factor V Leiden Genetic Health Risk Test for Hereditary Thrombophilia is to be performed using the Tecan Evo and Illumina iScan instruments. GenomeStudio is a modular software application that is used to view and analyze genotypic data obtained from the Illumina iScan System and based on the cluster definition file. AncestryDNA GHR software conducts a variety of control checks on the file, resulting in a final genotype profile for each sample. These data are used to generate test reports on a user's genotype and associated risk of disease.	The 23andMe PGS Genetic Health Risk Tests for Hereditary Thrombophilia is to be performed using the Tecan Evo and Illumina iScan instruments. GenomeStudio is a modular software application that is used to view and analyze genotypic data obtained from the iScan. Coregen software conducts a variety of control checks on the file, resulting in a final genotype profile for each sample. These data are used to generate test reports on a user's genotype and associated risk of disease.

	AncestryDNA Factor V Leiden Genetic Health Risk Test	**23andMe Personal Genome Service Test (Predicate Device)**
Classification	Class II	Same
Type of Test	Qualitative in vitro molecular diagnostic system	Same
Measurand	Factor V Leiden c.1601G>A variant in the F5 gene	Factor V Leiden variant in the F5 gene, and the Prothrombin G20210A variant in the F2 gene
Sample Preparation Method	DNA extraction	Same
Analytical Sensitivity	The performance requirement for the AncestryDNA GHR has been set at a minimum of 1.53 ng/µL DNA and maximum of 50 ng/µL DNA.	The performance requirement for the PGS, has been set at a minimum of 15 ng/µL DNA and maximum of 50 ng/µL DNA.
Reproducibility/ Precision	100% correct genotype calls for all samples with a valid call across multiple sites, multiple days, using multiple operator teams, instrument combinations, and assay reagent lots tested with samples collected using multiple lots of the AncestryDNA Sample Collection Kit at both laboratory sites. Genotyping results produced 99.9% (2337/2340) replicates that were called correctly and 0.13% (3/2340) replicates that did not pass quality control acceptance criteria. Samples with failed quality control (FQC) on the first run are re-tested per laboratory SOPs.	100% correct genotype calls for all samples with a valid call across multiple days, operator teams, instruments, and reagent lots at both laboratory sites. Genotyping results produced 98.0% (1852/1890) replicates that were called correctly and 2.01% (38/1890) replicates that did not pass quality control acceptance criteria. Samples with failed quality control (FQC) on the first run are re-tested per laboratory SOPs; an anticipated rate of samples with 2 FQCs based on the reproducibility study data of cell line samples is 0.04% (0.020 x 0.020).
Endogenous Interfering Substances	N = 4 endogenous agents were tested in saliva: salivary α-amylase, hemoglobin, IgA, and total protein. There was no impact on test performance with all interferents tested.	N = 4 endogenous agents were tested in saliva: salivary α-amylase, hemoglobin, IgA, and total protein. There was no impact on test performance with all interferents tested.
Exogenous Interfering Substances	N = 6 exogenous agents were tested in saliva samples collected after performing the following actions: eating food containing beef, eating food not containing beef, drinking	N = 6 exogenous agents were tested in saliva samples collected after performing the following actions: eating food containing beef, eating food not containing beef, drinking

	AncestryDNA Factor V Leiden Genetic Health Risk Test	23andMe Personal Genome Service Test (Predicate Device)
	alcohol, chewing gum, using mouthwash, and smoking. There was no impact on test performance at the 30 minute timepoint with all interferents tested.	alcohol, chewing gum, using mouthwash, and smoking. There was no impact on test performance at the 30 minute timepoint with all interferents tested.
Microbial Interfering Substances	N = 5 microbial agents were tested in saliva: *Staphylococcus epidermis*, *Streptococcus mutans*, *Lactobacillus casei*, *Actinomyces odontolyticus*, and *Candida albicans*. There was no impact on performance with all interferents tested.	From 23andMe Personal Genome Service Carrier Screening Test for Bloom Syndrome (DEN140044): N = 5 microbial agents were tested in saliva: *Staphylococcus epidermis*, *Streptococcus mutans*, *Lactobacillus casei*, *A.*, and *Candida albicans*. There was no impact on performance with all interferents tested.
Comparison with Sanger Bi-directional Sequencing	Overall agreement was 100% (198/198) with bi-directional sequencing.	Overall agreement was 100% (203/203) with bi-directional sequencing.
Clinical Performance	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."	The minor variant frequency for Factor V Leiden in individuals of European descent reported in published literature is 3%-15%; technical (analytical) positive predictive values for 23andMe PGS test results of CT and TT are ≥ 99.5% and ≥ 99.1 % correspondingly. The minor variant frequency for Prothrombin G2021 0A in individuals of European descent reported in published literature is 1 %--3%; technical (analytical) positive predictive values for 23andMe PGS test results of AG and AA are ≥ 98.6% and ≥ 97.2% correspondingly.
DIFFERENCES		
Sample Collection Device	AncestryDNA Saliva Collection Kit (K192947)	Oragene Dx Ogd-500.001 (K141410)
Interfering Mutations	The potential interfering mutations include rs770011773, rs773367113,	For Factor V Leiden the potential interfering mutations include

	AncestryDNA Factor V Leiden Genetic Health Risk Test	23andMe Personal Genome Service Test (Predicate Device)
	and rs143663052. The impact of these mutations on the performance of the assay has not been evaluated.	rs760488939 and rs763859650. The impact of these mutations on the performance of the assay has not been evaluated.

L. STANDARDS/GUIDANCE DOCUMENTS REFERENCED

- Special Controls for Genetic Health Risk Assessment System, as detailed in 21 CFR 866.5950.
- CLSI Guideline EP07-A3, Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition.
- CLSI Guideline EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; 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 Factor V Leiden GHR Test is performed by CLIA-certified laboratories using the BeadChip v10 assay (Illumina Infinium HumanOmniExpress-24 format chip) on the Illumina Infinium Platform. Samples collected using the AncestryDNA Saliva Collection Kit are delivered to laboratories for testing and analysis. DNA from saliva is quantified and samples with DNA concentrations greater than or equal to the limit of detection (1.53 ng/µL) and less than or equal to the maximum DNA concentration (50 ng/µL) are eligible for further processing. These samples are fragmented and captured on a bead array by hybridization to immobilized variant-specific primers, followed by extension with hapten-labeled nucleotides. The primers hybridize adjacent to the variants and are extended with a single nucleotide corresponding to the variant allele. The incorporated hapten-modified nucleotides are detected by adding fluorescently labeled antibodies in several steps to amplify the signals. The Tecan Evo is used in extraction and processing of the DNA, and the Illumina iScan is used to scan and quantify the results. Genotypes are determined using the GenomeStudio software package and delivered to Ancestry Genomics for analysis using the AncestryDNA GHR software.

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 studies, clinical studies and genotyping using the AncestryDNA Factor V Leiden GHR 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 calculate call rates. The data was then delivered to Ancestry Genomics where AncestryDNA 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, instrument combinations, and assay critical reagent lots tested with samples collected using multiple lots of the AncestryDNA Saliva Collection Kit (SCK). Execution of the study protocol and genotyping using the AncestryDNA Factor V Leiden GHR Test was performed at two (2) CLIA-certified laboratories (Lab 1 and Lab 2) on the Illumina Infinium array platform by six different operator teams (3 per laboratory) on eight instrument combinations (4 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 (9) donors provided 19 saliva samples into three lots of AncestryDNA SCKs. This study was performed over multiple days with three critical assay reagent lots for the AncestryDNA Factor V Leiden GHR Test evaluated in the Lab 1 and one critical assay reagent lot in the Lab 2 (Multi-Sample Amplification Master Mix (MSM), Fragmentation Solution (FMS), BeadChip, XP1 stain, and Two-Color Extension Master Mix (EML)). 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 sample call rate (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. For the intermediate precision, each of three operator teams tested each of the nine donor DNA samples in singlicate 20 times over five non-consecutive dates for each of three critical reagent lots,

across four different instrument combinations. For the within-run repeatability, each donor DNA sample was genotyped an additional four times on one plate (batch). Testing for this batch was performed by one operator team using one critical reagent lot and one instrument combination within a single day.

Summary of Lab 1 Within-Laboratory Testing Results

Genotype	Total Number of Replicates	Number of Concordant Calls	Number of "No-Calls"	Number of Call Rate QC Failures	FQC (%)
GG	540	540	0	0	0.00
GA	540	537	0	3*	0.56
AA	540	540	0	0	0.00
Total	**1620**	**1617**	**0**	**3**	**0.19**

*All three (3) QC failures were from the same donor

Results from the within-run repeatability study are shown in the table below. There were no genotyping repeats.

Within-Run Repeatability Results from Lab 1

Genotype	Total number of replicates	Number of concordant calls	Number of "No-Calls"	Number of call rate QC failures	FQC (%)
GG	15	15	0	0	0.00
GA	15	15	0	0	0.00
AA	15	15	0	0	0.00
Totals	**45**	**45**	**0**	**0**	**0.00**

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 using one critical reagent lot with four different instrument combinations.

Summary of Lab 2 Within-Laboratory Testing Results

Genotype	Total Number of Replicates	Number of Concordant Calls	Number of "No-Calls"	Number of Call Rate QC Failures	FQC (%)
GG	180	180	0	0	0.00
GA	180	180	0	0	0.00
AA	180	180	0	0	0.00
Total	**540**	**540**	**0**	**0**	**0.00**

Inter-laboratory Reproducibility

For the inter-laboratory reproducibility study, two saliva samples 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 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).

The final genotyping results for the inter-laboratory reproducibility study (n=2) across two instrument combinations, six operator teams, and two different reagent lot combinations (n=1 per lab) 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

Genotype	Total Number of Replicates		Number of Concordant Calls		Number of "No-Calls"		Number of Call Rate QC Failures		FQC (%)	
	Lab 1	***Lab 2***	***Lab 1***	***Lab 2***	***Lab 1***	***Lab 2***	***Lab 1***	***Lab 2***	***Lab 1***	***Lab 2***
GG	27	27	27	27	0	0	0	0	0.00	0.00
GA	27	27	27	27	0	0	0	0	0.00	0.00
AA	27	27	27	27	0	0	0	0	0.00	0.00
Totals	**81**	**81**	**81**	**81**	**0**	**0**	**0**	**0**	**0.00**	**0.00**

Results by Site and Operator Team

The final genotyping results by site per operator team per genotype across four reagent lot combinations, eight different instrument configurations, and 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 Team 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***

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 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 Team 2	**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 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 a single donor.

Results by Site and Instrument Combination

The final genotyping results by site per instrument combination per genotype across four reagent lot combinations, six different operator teams, and three AncestryDNA SCK lots is 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 Instrument Combination Results

Site/Instrument Combination	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-Instrument combination A	AA	168	168	0	0	0.00
	GA	168	168	0	0	0.00
	GG	168	168	0	0	0.00
Total		***504***	***504***	***0***	***0***	***0.00***
Lab 1-Instrument combination B	AA	135	135	0	0	0.00
	GA	135	134	0	1	0.74
	GG	135	135	0	0	0.00

Site/Instrument Combination	Expected Genotype	Total Number of Replicates	Number of Concordant Calls	Number of "No-Calls"	Number of Call Rate QC Failures	Proportion of FQC (%)
Total		***405***	***404***	***0***	***1***	***0.25***
Lab 1-Instrument combination C	AA	135	135	0	0	0.00
	GA	135	133	0	2	1.48
	GG	135	135	0	0	0.00
Total		***405***	***403***	***0***	***2***	***0.49***
Lab 1-Instrument combination D	AA	135	135	0	0	0.00
	GA	135	135	0	0	0.00
	GG	135	135	0	0	0.00
Total		***405***	***405***	***0***	***0***	***0.00***
Lab 2 -Instrument combination A	AA	72	72	0	0	0.00
	GA	72	72	0	0	0.00
	GG	72	72	0	0	0.00
Total		***216***	***216***	***0***	***0***	***0.00***
Lab 2 -Instrument combination B	AA	45	45	0	0	0.00
	GA	45	45	0	0	0.00
	GG	45	45	0	0	0.00
Total		***135***	***135***	***0***	***0***	***0.00***
Lab 2 -Instrument combination C	AA	45	45	0	0	0.00
	GA	45	45	0	0	0.00
	GG	45	45	0	0	0.00
Total		***135***	***135***	***0***	***0***	***0.00***
Lab 2 -Instrument combination D	AA	45	45	0	0	0.00
	GA	45	45	0	0	0.00
	GG	45	45	0	0	0.00
Total		***135***	***135***	***0***	***0***	***0.00***
All instruments' totals combined	**GG, GA, AA**	**2,340**	**2,337**	**0**	**3**	**0.13**

Results by Site and Factor V Leiden Reagent Lot Combination

The final genotyping results by site per critical reagent lot combination per genotype across eight instrument combinations, six different operator teams and three AncestryDNA SCK lots. The number of genotype calls includes replicates that pass the QC call rate and have genotype concordance with the expected genotype as determined by bi-directional sequencing.

Site and Critical Reagent Lot Combination Results

Site/Reagent Lot Combination	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 1	AA	213	213	0	0	0.00
	GA	213	211	0	2	0.94
	GG	213	213	0	0	0.00
Total		***639***	***637***	***0***	***2***	***0.31***
Lab 1-Lot 2	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 1-Lot 3	AA	180	180	0	0	0.00
	GA	180	179	0	1	0.56
	GG	180	180	0	0	0.00
Total		***540***	***539***	***0***	***1***	***0.19***
Lab 2-Lot 1	AA	207	207	0	0	0.00
	GA	207	207	0	0	0.00
	GG	207	207	0	0	0.00
Total		***621***	***621***	***0***	***0***	***0.00***
All reagent lots' totals combined	**GG, GA, AA**	**2,340**	**2,337**	**0**	**3**	**0.13**

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 instrument combination A	504 (504)	100.00 (99.27 – 100.00)

Attribute	Concordant Replicates (total QC passing replicates)	Point Estimate Percent Agreement (%) (95% confidence interval)
Lab 1 instrument combination B	404 (404)	100.00 (99.09 – 100.00)
Lab 1 instrument combination C	403 (403)	100.00 (99.09 – 100.00)
Lab 1 instrument combination D	405 (405)	100.00 (99.09 – 100.00)
Lab 2 instrument combination A	216 (216)	100.00 (98.31– 100.00)
Lab 2 instrument combination B	135 (135)	100.00 (97.30 – 100.00)
Lab 2 instrument combination C	135 (135)	100.00 (97.30 – 100.00)
Lab 2 instrument combination D	135 (135)	100.00 (97.30 – 100.00)
All instrument combinations	2,337 (2,337)	100.00 (99.84 – 100.00)
Lab 1 reagent lot 1	637 (637)	100.00 (99.42 – 100.00)
Lab 1 reagent lot 2	540 (540)	100.00 (99.32 – 100.00)
Lab 1 reagent lot 3	539 (539)	100.00 (99.32 – 100.00)
Lab 2 reagent lot 1	621 (621)	100.00 (99.41 – 100.00)
All reagent lot combinations	2,337 (2,337)	100.00 (99.84 – 100.00)
Within-run precision at Lab 1	1617 (1617)	100.00 (99.77 – 100.00)
Within-run precision at Lab 2	540 (540)	100.00 (99.32 – 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)
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, using multiple instrument combinations, and multiple reagent lots. 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 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)

Controls
The AncestryDNA Factor V Leiden Genetic Health Risk Test uses one (1) control material, which serves as both the sample processing control and the reproducibility control. The control material is genotyped on the Illumina BeadChip according to routine SOPs at the laboratory. Each new lot of the control is tested by comparison with reference BeadChip genotype results.

The control was tested for shelf life stability when stored at 2 to 8°C. This study is ongoing and performance will be evaluated against the pre-established acceptance criteria at 3-months and 4-months post-manufacturing. The interim data shows that the control is stable for a minimum of 1 month post-manufacturing.

The control was also tested for in-use stability. Data shows that the control has an in-use stability of 30 days from first opening.

Reagent Stability
This study is ongoing, and performance will be evaluated against the pre-established acceptance criteria at 3-months, 6-months, 12-months, and 13-months post-manufacturing. The interim data shows that the critical assay reagents are stable for a minimum of 2 months post-manufacturing.

The storage condition and temperature for the critical reagents (all are single use) are listed in the table below.

Storage conditions and temperatures for the critical assay reagents

Critical Reagent	Storage condition	Storage temperature
MSM	Frozen	-15°C to -25°C
FMS	Frozen	-15°C to -25°C
BeadChip	Refrigerated	2 to 8°C
X-Stain Plate	Frozen	-15°C to -25°C
EML	Frozen	-15°C to -25°C

d. Analytical Sensitivity

Two studies were conducted to determine Analytical Sensitivity, one using saliva samples and one using cell line samples.

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 using three (3) lots of critical reagents. 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
For the study using saliva samples, to establish the Limit of Blank (LoB), blanks consisting of molecular grade water and the standard volume of DNA stabilizing solution from the AncestryDNA SCK were extracted, quantified, and genotyped using three lots of critical reagents.

For the cell line study, blanks consisting of molecular grade water were quantified and genotyped using three lots of critical reagents.

Limit of Detection Test Method

For the study using saliva, samples were collected from 15 donors with known Factor V Leiden genotypes as determined using bi-directional sequencing: five 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, two (2) replicates per critical reagent lot (n=3) using the AncestryDNA Factor V Leiden GHR Test. Testing was performed under the standard protocol for the AncestryDNA GHR Test, except that each donor sample in the series was genotyped in duplicate per critical reagent lot. A total of 450 replicates were tested in the LoD study.

For the study using cell lines, four (4) cell lines with known Factor V Leiden genotypes as determined using bi-directional sequencing were used. DNA from each of the cell lines was diluted to generate one (1) four-fold dilution and six (6) two-fold dilutions. In addition, a neat sample was used for a total of eight (8) samples in each dilution series. The samples and water blanks were quantified by UV-Vis spectroscopy to determine DNA concentration and purity (A260/A280). A total of 27 replicates of each cell line dilution series were genotyped using the AncestryDNA Factor V Leiden GHR Assay; three (3) replicates per critical reagent lot (n=3) on three (3) testing days for a total of 216 data points per cell line. A total of 855 blank replicates were genotyped using the AncestryDNA Factor V Leiden GHR Assay: 95 replicates per critical reagent lot (n=3) on three (3) testing days. Testing was performed by one (1) operator team using one (1) instrument line.

Based on both studies using saliva samples and cell line samples:

- 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, microbial DNA, and mutational interferents 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
	2.05 mg/mL human serum albumin

A total of ten 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 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 (see above table). 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. Two lots of the assay reagents were used during the execution of the study. 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 each endogenous interferent, the acceptance criteria of ≥ 90% agreement with true variant status for all samples that had passed quality control had been met. For all samples where the control samples passed QC, the concordance for all interfering substances was 100%, meeting the acceptance criterion of ≥ 95% agreement with true variant status for all controls and endogenous substances 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 their effect on the performance of the AncestryDNA Factor V Leiden GHR Test. The exogenous interference study included samples from non-smokers and smokers. The study was performed with nine lots of assay reagents. 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 chicken	12	3	3	108
Drinking alcohol	12	3	3	108
Using mouthwash	12	3	3	108
Eating 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%. This 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 (GG),
- One cell line was Factor V Leiden heterozygous (GA), and
- One cell line was Factor V Leiden homozygous rare (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). One lot of reagent was used during the execution of the study.

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)

Mutational Interference

Nucleotide mutations were evaluated to determine the impact on the performance of the AncestryDNA Factor V Leiden GHR Test. An *in silico* analysis was performed to identify nucleotide mutations that could potentially interfere with the AncestryDNA Factor V Leiden GHR Test within the genomic region that is one probe length (50 base pairs (bp)) downstream of the Factor V Leiden mutation (rs6025; NC_00000l.11: g.169549811G>A; NM_000130.4: c.1601G>A). The genome aggregation database (gnomAD database) was utilized to search for mutations in this region. Only mutations that have been observed in more than one individual were evaluated. Three potentially interfering mutations were identified as summarized in below. These mutations are located within one (1) probe length of the Factor V Mutation and have an allele count >1. The impact of these mutations on the performance of the assay has not been evaluated.

Summary of Identified Potentially Interfering Mutations

rsID	Chromosome Position	Transcript Consequence	Annotation	Allele Count	Allele Frequency
rs770011773	g.169549812	c.1600C>T	Stop Gained	3	0.00001062
rs773367113	g.169549835	c.1577G>A	Missense Variant	2	0.000007073
rs143663052	g.169549848	c.1564C>G	Missense Variant	14	0.00004951

Two (2) mutations that were classified as potentially interfering in the predicate submission (DEN160026), rs760488939 and rs763859650, will not interfere in the AncestryDNA Factor V Leiden GHR Test since their genomic location is greater than 50 bp (one probe length) from rs6025. These potentially interfering mutations cannot be empirically tested due to the absence of independent probes on the array.

f. Assay Cut-off

Not Applicable.

g. Specimen Stability

Saliva samples for testing are collected with the AncestryDNA Saliva Collection Kit. See K192947 for sample stability information.

h. Shipping Stability

Saliva samples for testing are shipped in the AncestryDNA Saliva Collection Kit. See K192947 for sample shipping stability information.

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%. Of 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 distribution of the 11 FQC samples by genotype

Distribution of the 11 FQC samples by genotype

Genotype	Total Number of Samples	Number of FQC	FQC (%)	95% Confidence Interval
GG	73	4	5.5	1.5 - 13.4
AA	67	3	4.5	0.9 - 12.5
GA	69	4	5.8	1.6 - 14.2

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

AncestryDNA Factor V Leiden GHR Genotypes	Bi-directional Sequencing Genotypes			Total
	GG	GA	AA	
GG	69	0	0	69
GA	0	65	0	65
AA	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

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 rate point estimate of no-call events and 95% confidence intervals below were 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.

Estimated Rate of No-Call Events for the AncestryDNA Factor V Leiden GHR Test

Event	Observed/Total	Rate Point Estimate	95% Confidence Interval
No-call	0/198	0%	0 – 1.8%

The calculated overall percent agreement and 95% confidence intervals below were 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. This result met the predefined protocol acceptance criterion of ≥ 99% agreement with true variant determination overall and per genotype tested.

b. Matrix Comparison

Not applicable.

3. Clinical Studies

a. Clinical Performance

Odds ratios

Odds ratios are available in the meta-analyses performed by Simone *et al.* (2013). Odds ratios adjusted for age and sex using logistic regression and 95% confidence intervals for each variant status are given below:

Factor V Leiden variant status	Odds ratio (95% confidence interval)
One copy, heterozygote	4.22 (3.35 – 5.32)
Two copies, homozygote	5.45 (6.79 – 19.29)

Likelihood ratios and post-test risk

Likelihood ratios (*LR*) were obtained using the raw distribution data from the meta-analyses in Simone *et al.* (2013).

Post-test risk (R_{post}) was calculated from the likelihood ratios and using a pre-test risk (R_{pre}) of 11% from the 2018 ACMG Venous thromboembolism laboratory testing standard (Zhang, 2018). Likelihood ratios and 95% confidence intervals calculated using a normal approximation are listed in the table below along with the post-test risk calculated using the relationship $R_{post}/(1 - R_{post}) = LR\ [R_{pre}/(1 - R_{pre})]$:

FVL variant count (genotype)	Case/control distribution	LR	95% confidence interval	Post-test risk
0 variants (homozygous common / wild type)	1,758 cases and 1,201 controls among 2,959 Factor V Leiden carriers. 7,323 cases and 16,312 controls among 23,635 wild type individuals.	0.87	0.86 – 0.88	10%
1 variant (heterozygous)	1,758 cases and 1,201 controls among 2,959 Factor V Leiden carriers. 7,323 cases and 16,312 controls among 23,635 wild type individuals.	2.82	2.64 – 3.02	26%
2 variants (homozygous rare)	92 cases and 24 controls among 116 Factor V Leiden homozygous individuals. 4,524 cases and 11,643 controls among 16167 wild type individuals.	9.69	6.19 – 15.16	54%

Note that the likelihood ratio lower bounds for heterozygotes and homozygotes are greater than one, indicating that the post-test risk is significantly greater than the pre-test risk.

References:
1. Zhang, Shulin, et al. "Venous thromboembolism laboratory testing (factor V Leiden and factor II c.* 97G> A), 2018 update: a technical standard of the American College of Medical Genetics and Genomics (ACMG)." Genetics in Medicine 20.12 (2018): 1489.
2. Simone, Benedetto, et al. "Risk of venous thromboembolism associated with single and combined effects of Factor V Leiden, Prothrombin 20210A and Methylenetethraydrofolate reductase C677T: a meta-analysis involving over 11,000 cases and 21,000 controls." (2013): 621-647.

b. User Comprehension Studies

Ancestry Genomics sponsored two studies to assess user comprehension of the AncestryDNA Factor V Leiden GHR Test's Genetic Health Risk (GHR) reports.

The first user comprehension study, Study 1, was designed across all report types using a post- test administered questionnaire. Hence, a second study, Study 2, was designed to assess comprehension across the two most challenging reports and evaluate comprehension using a pre-test and post-test administered questionnaire.

In both studies, a Genetic Risk Education Module was developed to provide eligible participants with a brief overview of genetics in order to prepare them for review of the GHR report and explain the significance of genetic risk reports. 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 U.S. Census geographic regions: Northeast, Midwest, South, and West.

In Study 1, a total of 378 individuals were enrolled as participants into each of four study arms, one for each of the GHR report types:
1. 0 Variants Identified
2. 1 Variant Identified
3. 2 Variants Identified
4. Result Not Determined

In Study 2, a total of 213 individuals were enrolled as participants into each of two study arms, one for each of the following two GHR report types:
1. 1 Variant Identified
2. Result Not Determined

Both user comprehension studies were performed on the different types of the GHR reports developed using representative samples of the materials below ("supplemental materials").

Supplemental Materials
- Education Module including definition of terms,
- Pre-purchase page,
- Frequently Asked Questions, and
- Technical Details.

The representative samples were developed by Ancestry Genomics based on FDA guidance for medical device patient labeling and designed for readability no higher than 8th grade reading level using a Flesch-Kincaid Readability Test. The representative samples were reviewed by a Certified Genetic Counselor to confirm that the materials tested accomplished the following:
- Defined the target condition being tested and related symptoms,
- Explained the intended use and limitations of the test,
- Explained the relevant ethnicities in regard to the variant tested,
- Explained genetic health risks and relevance to the user's ethnicity, and
- Assessed participants' ability to understand the following comprehension concepts: the test's limitations, purpose, appropriate follow-up action, test results, ethnic relevance and other risk factors that may have an impact on the test results.

In Study 1, which was conducted in-person, participants were observed discretely, and each interview session was administered by a trained interviewer/moderator using a series of predefined questions. Participants were given a time limit from start of report review to completion of the comprehension survey. There was a 100% response rate for all questions for all participants included in the data analysis. Failing to respond to numerous items in the questionnaire was one of the criteria used to identify participants for exclusion from data analysis.

In Study 2, which was conducted via live televideo interviews, participants were observed via live video, and each interview session was administered online by a trained interviewer/moderator using a series of predefined questions. Participants were first administered a pre-test questionnaire prior to being shown any of the representative samples. Participants were then given a time limit from start of report review to completion of the post-test questionnaire. There was a 100% response rate for all questions for all participants included in the data analysis. Failing to respond to numerous items in the questionnaire was one of the criteria used to identify participants for exclusion from data analysis. All pre-test questions were repeated again in the post-test questionnaire to assess pre-test vs post-test improvement.

The table below summarizes comprehension scores in Study 1 for each core concept evaluated by report type and for the overall comprehension rate. The average comprehension rates per core comprehension concept range from 90.7% to 97.4% for all subjects who participated in the study.

Study 1 - Overall User Comprehension Rates for Factor V Leiden GHR Reports

Core Concept	Comprehension Rates by GHR Report Type (%)				Overall Comprehension Rates
	0 Variant	1 Variant	2 Variant	Result Not Determined	
Appropriate Follow-Up Action	95.8	97.7	100	95.6	97.4%
Ethnic Relevance	98.9	89.5	97.2	N/A	95.5%
Other Risk Factors	89.6	94.2	92.5	92.2	92.1%
Limitations of Test	88.5	89.5	98.1	88.9	91.5%
Purpose of Test	89.0	95.3	93.4	93.3	92.7%
Results of Test	93.8	83.7	93.4	91.1	90.7%
Total Number of Reports	N = 96	N = 86	N = 106	N = 90	N = 378

The table below summarizes comprehension scores in Study 2 for each core concept evaluated by report type and for the overall comprehension rate. The average comprehension rates per core comprehension concept range from 90.9% to 99.1% for all subjects who participated in the study.

Study 2 - Overall User Comprehension Rates for Factor V Leiden GHR Reports

Core Concept	Comprehension Rates by GHR Report Type (%)		Overall Comprehension Rates
	1 Variant	Result Not Determined	
Appropriate Follow-Up Action	94.2	98.6	96.5%
Ethnic Relevance	98.1	97.3	97.7%
Other Risk Factors	98.1	93.6	95.8%
Limitations of Test	93.7	98.6	96.2%
Purpose of Test	98.5	99.6	99.1%
Results of Test	88.8	92.7	90.9%
Total Number of Reports	N = 103	N = 110	N = 213

The acceptance criteria were met for both User Comprehension Studies which stated ≥ 90% comprehension score for each of the domains evaluated: test results, test purpose, test limitations, appropriate follow-up action, ethnic relevance, and other risk factors that may impact the test results. In addition, the overall comprehension score across all GHR reports was 93.2% for Study 1 and 96% for Study 2.

In addition, Study 2 was able to show that there was an improvement in comprehension between pre-test versus post-test. The table below shows the pre-test versus post-test statistical comparison by comprehension concept.

User Comprehension by Comprehension Concept Pre- to Post-Test Statistical Comparison

Comprehension Concept	Pre-test (%)	Post Test (%)	% Improvement	p-value
Purpose	89.0	99.1	10.1	<0.001
Other Risk Factors	91.1	95.8	4.7	0.002
Ethnic Relevance	95.8	97.7	1.9	0.117
Limitations	87.7	96.2	8.5	<0.001

c. Expected Values/Reference Range

Not applicable.

O. INSTRUMENT NAME

Illumina iScan BeadChip scanner with GenomeStudio software

P. SYSTEM DESCRIPTION

1. Modes of Operation:

   The Illumina iScan is a table top laser-based, high-resolution optical imaging system that produces genotype information for up to 4 beadchips/beadchip carrier. Carriers are loaded into the instrument through an Autoloader2.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
   Yes __X__ or No _____

   Level of Concern:
   Moderate

   Software Description:
   Illumina iScan System with iScan Control Software and Genome Studio performs the following:
   - iScan Control Software drives the iScan hardware in Beadchip scanning and image data generation.
   - GenomeStudio software allows viewing and analyzing of genotypic data obtained from the iScan. Processing includes primary data analyses, such as raw data normalization, clustering, and genotype calling. To ensure data quality, the software performs internal controls and data quality control checks.
   - AncestryDNA GHR software takes raw data and putative genotype calls from the PED file received from the laboratory and generates the final analytical genotype information for each sample.

Revision Level History:
A software revision history record for the AncestryDNA GHR software was acceptable.

Unresolved Anomalies:
There are no known unresolved anomalies associated with the system software.

EMC Testing:
Not applicable.

3. Specimen Identification

Users must register their saliva collection kit, linking their saliva sample to a secure online account with a valid email address through a unique activation code, in order to use the test. The activation code is matched to records of kits shipped to consumers to ensure it is a valid kit. A timestamp of the user completing the entries to activate the kit is recorded.

4. Specimen Sampling and Handling

Saliva samples should be collected using the AncestryDNA Saliva Collection Kit. The recommended volume of saliva is 1 mL. Saliva is collected directly by the user spitting into the provided Saliva Collection Tube via the pre-installed funnel. After providing saliva, the user is instructed to remove the funnel and screw on tightly the provided cap. Affixing the cap by screwing on releases the stabilization solution. The saliva sample 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.

5. Calibration

Calibration and calibration verification procedures are established to demonstrate continued accuracy of the test systems.

6. Quality Control

The AncestryDNA Factor V Leiden Genetic Health Risk Test uses one (1) control material, which serves as both the sample processing control and the reproducibility control. The control material is genotyped on the Illumina BeadChip according to routine SOPs at the laboratory. Each new lot of the control is tested by comparison with reference BeadChip genotype results.

The sample processing control is run on every sample genotyping plate and the reproducibility control is run approximately once per week.

Q. OTHER SUPPORTIVE INSTRUMENT PERFORMANCE CHARACTERISTICS DATA NOT COVERED IN SECTION N, "PERFORMANCE CHARACTERISTICS," ABOVE

Refer to the AncestryDNA Saliva Collection Kit 510(k) (K192947) for saliva collection device details and study results.

R. PROPOSED LABELING

The labeling satisfies the requirements of 21 CFR Parts 801 and 809 as well as the Special Controls for a Genetic Health Risk Assessment System.

S. CONCLUSION

The results of the analytical studies submitted in this 510(k) Premarket Notification are complete and demonstrate that the AncestryDNA Factor V Leiden Genetic Health Risk Test meets the established specifications necessary for consistent performance during the intended use of reporting the Factor V Leiden variant in the F5 gene. The results support a decision that the AncestryDNA Factor V Leiden Genetic Health Risk Test is substantially equivalent to the predicate.