510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K241456

B Applicant

Targeted Genomics, LLC

C Proprietary and Established Names

GlutenID Celiac Genetic Health Risk Test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PTA	Class II	21 CFR 866.5950 - Genetic Health Risk Assessment System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Variants associated with HLA-DQ2.5, HLA-DQ8, HLA- DQ7 and HLA-DQ2.2 haplotypes, in genomic DNA obtained from a human saliva sample.

C Type of Test:

Over-the-counter (OTC) genetic testing service, qualitative single nucleotide polymorphism (SNP) genotyping using next generation sequencing (NGS)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The GlutenID Celiac Genetic Health Risk Test uses qualitative genotyping to detect clinically relevant variants in genomic DNA isolated from saliva collected from individuals 18 years of age or older with ORAcollect Dx OCD-100 for the purpose of reporting and interpreting Genetic Health Risks (GHR).

The GlutenID Celiac GHR Test is indicated for reporting of one variant associated with the HLA-DQ2.5 haplotype, one variant associated with the HLA-DQ8 haplotype, one variant associated with the HLA-DQ7 haplotype, and three variants associated with the HLA-DQ2.2 haplotype. The report describes if a person has variants linked to haplotypes associated with an increased risk for developing celiac disease, but it does not describe a person's overall risk of developing celiac disease. This report is most relevant for people of European descent.

C Special Conditions for Use Statement(s):

- For over-the-counter (OTC) use.
- The test is intended for users ≥ 18 years old.
- This test is not a substitute for visits to a genetic counselor or healthcare provider. It is recommended that the user consults with a genetic counselor or healthcare provider if there are any questions or concerns about the results.
- The test does not detect all genetic variants related to celiac disease. The absence of a variant tested does not rule out the presence of other genetic variants that may be celiac disease related.
- The test does not diagnose any specific health conditions, determine medical treatment or other medical intervention, or tell the user anything about their current state of health. Only a healthcare provider can diagnose celiac disease. Results from this test should not be used solely to make medical decisions.
- If the user has other risk factors for the celiac disease, they should discuss this with a genetic counselor or healthcare provider.
- A user's race, ethnicity, age, sex, and lifestyle may affect how the genetic results are interpreted.
- The laboratory may not be able to process a patient's sample due to low DNA quantity/quality. This is estimated to occur with a frequency of less than 1%. If this happens, the user will receive an email notification.
- Subject to meeting the limitations contained in the special controls under regulation 21 CFR 866.5950.

D Special Instrument Requirements:

The GlutenID Celiac GHR Test is to be performed on the Illumina MiSeqDx instrument (DEN130011).

The GlutenID Celiac GHR Test software is comprised of two modules. The Pipeline module serves to process sequencing data from the Illumina MiSeqDx instrument into annotated variants. The Reporting module generates final reports for individuals based on the annotated variants results.

IV Device/System Characteristics:

A Device Description:

The GlutenID Celiac GHR Test is a direct-to-consumer DNA genetic test intended for OTC use. The test uses qualitative genotyping to detect clinically relevant genetic variants associated with the risk of developing celiac disease. The GlutenID Celiac GHR Test is indicated for reporting of one variant associated with the HLA-DQ2.5 haplotype, one variant associated with the HLA-DQ8 haplotype, one variant associated with the HLA-DQ7 haplotype, and three variants associated with the HLA-DQ2.2 haplotype as shown below:

Haplotype	SNP ID
HLA-DQ 2.5*	rs2187668
HLA-DQ8	rs7454108
HLA-DQ7	rs4639334
HLA-DQ2.2^	rs2395182
	rs7775228
	rs4713586

* DQ2.5 haplotype is commonly referred to as DQ2(cis)
^ DQ2.2 haplotype can be referred to as half-DQ2

The tests are conducted with genomic DNA isolated from human saliva sample collected in the ORAcollect Dx OCD-100 (cleared under K212745).

The GlutenID Celiac GHR Test is composed of:

(i) ORAcollect·Dx OCD-100 is intended for use in the collection of saliva samples.

(ii) GlutenID Celiac GHR Test is intended for the genetic analysis and detection of genetic variants associated with the HLA-DQ haplotypes using NGS.

(iii) GlutenID Pipeline Software is developed and validated on the Galaxy Platform as a modular workflow and data analysis application used to view and analyze genotyping data from the Illumina MiSeqDx. The GID Pipeline outputs base calling, read alignment, and variant identification.

(iv) The GlutenID Reporting Software is developed and validated to automate variant annotation from the technical data. It provides a Consolidated Report with predicted GlutenID genotype for each sample. Individual GlutenID Final Reports are automatically generated based on the predicted GlutenID from the Consolidated Report.

(v) GlutenID Celiac GHR website and result portal software is intended to provide the contents and the procedure to order and use the OTC Service.

A user's saliva is self-collected using ORAcollect Dx OCD-100 device which consists of a collection tube containing a stabilizing buffer solution. The collected sample is shipped to a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory for processing, testing and analysis. Human genomic DNA isolated from the saliva sample is isolated, processed and sequenced using NGS reagents on the Illumina MiSeqDx instrument. Following completion of a sequencing run, the data is analyzed using MiSeq integrated system software and genotyping results of the six celiac genetic risk variants are automatically processed into one of 15 associated GlutenIDs genotypes. The personalized final reports for each sample are generated using the GlutenID Reporting Software to provide information about the celiac disease genetic risk associated with their assigned genotypes.

The GlutenID Risk category for each of the 15 GlutenID genotypes are shown in the following table:

GlutenID Genotype	**Estimated Risk**	**Risk Category**
DQ2+DQ2	1 in 9 (8%)	Increased
DQ2+half-DQ2	1 in 9 (8%)	
DQ2+DQ8	1 in 12 (6%)	
DQ8+half-DQ2	1 in 25 (4%)	
DQ2+DQ7	1 in 35 (3%)	
DQ2.2+DQ7 [DQ2 (trans)]	1 in 35 (3%)	
DQ2 (cis)	1 in 35 (3%)	
DQ8+DQ8	1 in 100 (1%)	Low
DQ8	1 in 100 (1%)	
DQ8+DQ7	1 in 100 (1%)	
Half-DQ2+half-DQ2 (DQ2.2+DQ2.2)	1 in 210 (0.5%)	Not likely at risk
Half-DQ2 (DQ2.2)	1 in 210 (0.5%)	
DQ7+DQ7	1 in 1842 (0.05%)	
DQ7	1 in 1842 (0.05%)	
Non-celiac genetics (NCG)	1 in 2518 (0.05%)	

Note: DQ2(cis) refers to DQ2.5 haplotype, half-DQ2 refers to DQ2.2 haplotype, DQ7+DQ2.2 refers to DQ2(trans).

B Principle of Operation:

The GlutenID Celiac GHR Test is performed by a CLIA-certified laboratory. The test is a qualitative single nucleotide polymorphism (SNP) genotyping assay that is performed for each sample using NGS to detect the variants associated with the haplotypes HLA-DQ2.5, HLA-DQ8, HLA-DQ7, HLA-DQ2.2.

Genomic DNA is extracted from human saliva samples collected using ORAcollect·Dx OCD-100. Celiac disease associated SNP genomic regions of each DNA sample are amplified. This results in the generation of sample libraries that are transferred into a MiSeqDx reagent cartridge containing all required reagents. The cartridge is inserted into the MiSeqDx instrument which

performs cluster generation, sequencing, and data analysis. The raw data are uploaded onto the Galaxy Platform and analyzed using the GlutenID Pipeline Software. Genotyping results of the six celiac disease genetic risk variants are automatically processed into one of 15 associated GlutenID genotypes by the GlutenID Reporting Software. The GlutenID Celiac GHR Test interprets and categorizes each user's Gluten ID genotype into risk categories (Not likely at risk, Low risk, and Increased risk) based on the estimated lifetime risk of celiac disease.

V Substantial Equivalence Information:

A Predicate Device Name(s):

23andMe Personal Genome Service (PGS) Genetic Health Risk Test for Celiac Disease

B Predicate 510(k) Number(s):

DEN160026

C Comparison with Predicate(s):

Device & Predicate Device(s):	K241456	DEN160026 (Predicate)
Device Trade Name	GlutenID Celiac Genetic Health Risk (GHR) Test	23andMe Personal Genome Service (PGS) Genetic Health Risk Test for Celiac Disease
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The GlutenID Celiac Genetic Health Risk Test uses qualitative genotyping to detect clinically relevant variants in genomic DNA isolated from saliva collected from individuals 18 years of age or older with ORAcollect Dx OCD-100 for the purpose of reporting and interpreting Genetic Health Risks (GHR). The GlutenID Celiac GHR Test is indicated for reporting of one variant associated with the HLA-DQ2.5 haplotype, one variant associated with the HLA-DQ8 haplotype, one variant associated with the HLA-DQ7 haplotype, and three variants associated with the HLA-DQ2.2 haplotype. The report describes if a person has variants linked to haplotypes associated with an	The 23andMe Personal Genome Service (PGS) Test uses qualitative genotyping to detect the following clinically relevant variants in genomic DNA isolated from human saliva collected from individuals ≥18 years with the Oragene Dx model OGD-500.001 for the purpose of reporting and interpreting Genetic Health Risks (GHR). The 23andMe PGS Genetic Health Risk Report for Celiac Disease is indicated for reporting of a variant associated with the HLA-DQ2.5 haplotype. The report describes if a person has a haplotype associated with an increased risk of developing celiac disease, but it does not describe a person's overall risk for

	increased risk for developing celiac disease, but it does not describe a person's overall risk of developing celiac disease. This report is most relevant for people of European descent.	developing celiac disease. This report is most relevant for people of European descent.
Special Conditions for Use	• For over-the-counter (OTC) use. • The test is intended for users ≥ 18 years old. • This test is not a substitute for visits to a genetic counselor or healthcare provider. It is recommended that the user consults with a genetic counselor or healthcare provider if there are any questions or concerns about the results. • The test does not detect all genetic variants related to celiac disease. The absence of a variant tested does not rule out the presence of other genetic variants that may be celiac disease related. • The test does not diagnose any specific health conditions, determine medical treatment or other medical intervention, or tell the user anything about their current state of health. Only a healthcare provider can diagnose celiac disease. Results from this test should not be used solely to make medical decisions. • If the user has other risk factors for the celiac disease, they should discuss this with a genetic counselor or healthcare provider. • A user's race, ethnicity, age, sex, and lifestyle may affect how the genetic results are interpreted. • The laboratory may not be able to process a patient's sample due to low DNA quantity/quality. This is estimated to occur with a frequency of less than 1%. If this happens, the user will receive an email notification. • Subject to meeting the limitations contained in the special controls under regulation 21 CFR 866.5950.	• For over-the-counter (OTC) use. • 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. • The 23andMe PGS Genetic Health Risk Tests for Celiac disease, do not detect all genetic variants associated with the celiac disease. The absence of a variant tested does not rule out the presence of other genetic variants that may be disease related. • The test is intended for users ≥ 18 years old. • The test does not diagnose any specific health conditions. Results should not be used to make medical decisions. • 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%. • A user's race, ethnicity, age, and sex may affect how the genetic test results are interpreted. • Subject to meeting the limitations contained in the special controls under regulation 21 CFR 866.5950.

Type of Test	Qualitative genotyping for allelic variant determination through SNP detection	Same
Design	Software application that includes product information page, e-commerce (registration and order DNA kit), secure login, download genetic report	Same
Interpretation of Results	For OTC use. Specialized interpretation by a physician not required	Same
Specimen Type	Saliva	Same
Measurand	Genomic DNA	Same
Human Factors	User Comprehension Study	Same
Intended User	≥ 18 years old	Same
General Device Characteristic Differences		
Specimen Collection	ORAcollect Dx model OCD-100	Oragene Dx model OGD-500.001
Variants Detected	HLA-DQA1, HLA-DQB1 haplotypes DQ2.5, DQ8, DQ7, DQ2.2	HLA-DQA1, HLA-DQB1 haplotypes DQ2.5
Technology	Next generation sequencing (NGS)	Microarray genotyping
Instrument	Illumina MiSeqDx System	Tecan Evo and Illumina iScan
Software	The GlutenID Pipeline Software application is a modular software used to view and analyze genomic data from the MiSeqDx. The GlutenID Reporting software application conducts a variety of control checks on the file resulting in automated technical reporting of the sample's genotype profile. Final reports with associated disease risk are automatically generated from the technical report data.	GenomeStudio is a modular software application that is used to view and analyze genotype 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

VI Standards/Guidance Documents Referenced:

- CLSI EP12-Ed3, Evaluation of Qualitative, Binary Output Examination Performance
- Class II Special Controls: 866.5950 - Code of Federal Regulations Title 21
- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices (2005)
- General Principles of Software Validation Guidance for Industry and FDA Staff (2002)
- Off-The-Shelf Software Use in Medical Devices Guidance for Industry and Food and Drug Administration Staff (2019)

- Content of Premarket Submissions for Management of Cybersecurity in Medical Devices (2014)

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

The test performance was validated at the single site, and the results presented below met the pre-defined acceptance criteria outlined in the Special Controls of 21 CFR 866.5950. Information regarding samples that failed quality control (FQC) was also evaluated and presented in each study below.

1. Precision/Reproducibility:

An evaluation of precision of the GlutenID Celiac GHR Test was performed by testing 30 samples representing variants for each celiac genetic risk haplotypes: DQ2.2, n=5; DQ2.5, n=7; DQ7, n=4; DQ8, n=7; DQ2+DQ2.2, n=2; DQ2+DQ8, n=1; DQ2.2+DQ7 [DQ2(trans)], n=4. Testing included using three reagent lots, three MiSeqDx analyzers, two thermocyclers and three different operators. Each sample was run in three replicates per run, three runs per day for three non-consecutive days, for a total of 270 results. Each run combined different reagent lots, instruments, and operators at a single site. Relevant genotypes for the samples were confirmed by bi-directional Sanger sequencing. Number of correct calls, number of failed QC (FQC), % of FQC, and %correct calls were analyzed. The results are presented in the following table:

Haplotype	No. of Samples	No. of Replicates	No. of Correct Calls	No. of FQC	% of FQCs	% of Correct Calls
DQ2.2	5	45	45	0	0%	100%
DQ2.5	7	63	63	0	0%	100%
DQ7	4	36	36	0	0%	100%
DQ8	7	63	63	0	0%	100%
DQ2+DQ2.2	2	18	18	0	0%	100%
DQ2.2+DQ7 DQ2(trans)	4	36	36	0	0%	100%
DQ2+DQ8	1	9	9	0	0%	100%
Total	**30**	**270**	**270**	**0**	**0%**	**100%**

- No samples failed QC (FQC); percentage of FQC was 0% (0/270) with 95% confidence interval (CI): (0.0%; 1.4%).
- A total of 270 results were in the precision study across three lots, three operators, three days, and three machine combinations. Percentage of correct calls was 100% (270/270) with 95% CI: (98.6%; 100%).

2. DNA Extraction Precision Study

To evaluate the effect of the extraction step on samples used with GlutenID Celiac GHR Test, a separate DNA extraction study was conducted. Three saliva samples were self-

collected from each of 12 donors representing the four haplotypes (DQ2.5, n=6; DQ8, n=2; DQ7, n=2; DQ2.2, n=2). Two operators performed one independent DNA extraction on each of three non-consecutive days for a total of six runs of DNA extraction per subject. Each extracted DNA was tested with the GlutenID Celiac GHR Test in duplicate. A total of two GlutenID Celiac GHR Test runs were performed (Run 1 with the DNA extractions from operator 1 and Run 2 with the DNA extractions from operator 2) and the total number of results per subject was 12 (3 samples x 4 results/sample). The total number of DNA extractions was 72 with the total number of 144 over the course of the study (12 subjects x 2 operators x 3 days x 1 DNA extraction per operator per sample per day x 2 replicates). The results are summarized in the following table:

Haplotype	No. of Subjects	No. of Samples	No. of Extractions	No. of Replicates	No. of Correct Calls	No. of FQCs	% of FQCs	% of Correct Calls
DQ2.2	2	6	12	24	24	0	0%	100%
DQ2.5	6	18	36	72	72	0	0%	100%
DQ7	2	6	12	24	24	0	0%	100%
DQ8	2	6	12	24	24	0	0%	100%
Total	**12**	**36**	**72**	**144**	**144**	**0**	**0%**	**100%**

- No samples failed QC (FQC); %FQC was 0% (0/144) with 95% CI: (0.0%; 2.6%).
- Percentage of correct calls was 100% (144/144) with 95% CI: (97.4%; 100%)

3. Lot-to-Lot Precision

To evaluate the lot-to-lot imprecision for the GlutenID Celiac GHR Test, a study was conducted by three operators who prepared three different reagent lots to ensure three lots contained different manufacturing preparation of the critical reagents. Each operator tested a panel of six saliva DNA samples representing the DQ2, DQ2+DQ2, DQ8, DQ8+DQ8, and DQ2.2+DQ7 genotypes in triplicate using one of the three lots, resulting a total of 18 results (6 sample x 3 replicates) per lot and a total of 54 replicates (18 replicates x 3 lots). The results are summarized in the following table:

Haplotype	No. of Replicates	No. of Correct Calls			No. of FQCs			% of FQCs	% of Correct Calls
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3		
DQ2	9	3	3	3	0	0	0	0%	100%
DQ2+DQ2	9	3	3	3	0	0	0	0%	100%
DQ8	9	3	3	3	0	0	0	0%	100%
DQ8+DQ8	9	3	3	3	0	0	0	0%	100%
DQ2.2+DQ7	9	3	3	3	0	0	0	0%	100%
DQ2.2+DQ7	9	3	3	3	0	0	0	0%	100%
Total	**54**	**18**	**18**	**18**	**0**	**0**	**0**	**0%**	**100%**

- No samples failed QC (FQC); percentage of FQC was 0% (0/54) with 95% CI: (0.0%; 6.6%).
- Percentage of correct calls 100% (54/54) across three reagent lots with 95% CI: (93.4%; 100%)

4. Linearity:

Not applicable

5. Analytical Specificity/Interference:

Saliva samples collected using the ORAcollect Dx OCD-100 device were evaluated for the impact of endogenous, exogenous, smoking, and microbial substances, on the performance of the GlutenID Celiac GHR Test.

i. Endogenous Interference

An endogenous interference study was conducted to determine whether potential endogenous substances present during saliva collection affect the performance of the GlutenID Celiac GHR Test. Saliva samples were self-collected from five individuals (representing the four haplotypes DQ2.2, DQ2.5, DQ7, and DQ8). Samples were split and individual aliquots were spiked with the following:

- salivary amylase: 395 U/ml
- hemoglobin: 20 mg/mL
- IgA: 0.43 mg/mL
- Total protein: 2.67 mg/mL (composed of 0.185 mg/mL salivary amylase, 0.43 mg/mL IgA, and 2.05 mg/ml Human Serum Albumin)

Three replicates were tested for each aliquot. Each replicate was tested on the same day, with one lot of reagents, by one operator, using one set of instruments. The variant calls in the spiked samples were compared to the control samples. The results are summarized in the following table:

Haplotypes	No. of Replicates	No. of Correct Calls	No. of Incorrect Calls	No. of FQC	% of FQC	% Concordance with Control
DQ2.2	12	12	0	0	0%	100%
DQ2.5*	24	24	0	0	0%	100%
DQ7	12	12	0	0	0%	100%
DQ8	12	12	0	0	0%	100%
Total	**60**	**60**	**0**	**0**	**0%**	**100%**
*Includes two heterozygous samples						

No samples failed QC. All samples tested with the endogenous substances yielded 100% concordance with the control. None of the tested substances interfered with the GlutenID test at the concentrations tested.

ii. Exogenous Interference

Samples were self-collected by five individuals (representing the four celiac disease genetic risk haplotypes DQ2.2, DQ2.5, DQ7, and DQ8) at three collection times: before consuming an exogenous substance (baseline), immediately (0 minutes) after, and 30 minutes after. At each collection point, an aliquot of the saliva sample collected from

ORAcollect Dx sample collection device was processed according to the laboratory's SOP for DNA extraction. The testing conditions included:

- eating beef
- eating food other than beef
- drinking
- chewing gum
- using mouthwash

At each testing point, the samples were tested in triplicate, yielding a total of 225 results (5 donors x 5 conditions x 3 time points x 3 replicates). The combined results are summarized in the table below:

Haplotypes	No. of Replicates	No. of Correct Calls	No. of Incorrect Calls	No. of FQC	% of FQC	% Concordance
DQ2.2	45	44	0	1	2.2%	100%
DQ2.5*	90	85	0	5	5.6%	100%
DQ7	45	45	0	0	0%	100%
DQ8	45	44	0	1	2.2%	100%
Total	**225**	**218**	**0**	**7**	**3.1%**	**100%**
*Includes two heterozygous samples						

Out of a total of 225 replicates run, 7/225 (3.1%) failed QC (FQC). All seven had LowDP (sequencing depth coverage below the laboratory cutoff for the assay) and were collected after exposure to exogenous interfering substances. Of the failures, five were seen with DQ2.5, one with DQ8 and one with DQ2.2. Two failures came immediately after eating beef (one with DQ2.5 and one with DQ8), one (DQ2.5) immediately after drinking and one (DQ2.5) immediately after mouthwash. Three of the failures occurred 30 minutes after exposure to beef (one DQ2.2), non-beef food (one DQ2.5) and chewing gum (one DQ2.5). All FQC samples were repeated and yielded correct results on the second run.

Based on these results, it is recommended that collection of saliva samples occurs at least 30 minutes after eating, drinking, using mouthwash, and brushing teeth.

iii. Smoking

A study was performed to evaluate the effects of smoking before saliva collection on the performance of the GlutenID Celiac GHR Test. Five individuals (representing the four haplotypes DQ2.2 DQ2.5, DQ7, andDQ8) provided three samples each (baseline/control sample taken at least 60 minutes prior to smoking, samples collected immediately after smoking, and samples collected 30 minutes after smoking. The smoking samples were tested in triplicate for a total of 45 test samples (5 donors x 3 time points x 3 replicates). The results are summarized in the following table:

Haplotypes	No. of Replicates	No. of Correct Calls	No. of Incorrect Calls	No. of FQC	% of FQC	% of Concordance
DQ2.2	9	9	0	0	0%	100%
DQ2.5*	18	15	0	3	16.7%	100%
DQ7	9	9	0	0	0%	100%
DQ8	9	9	0	0	0%	100%
Total	**45**	**42**	**0**	**3**	**6.7%**	**100%**
*Includes two heterozygous samples						

Out of a total of 45 replicates run, three (6.7%) failed QC in this study. All three are with DQ2.5 and had DNA concentrations below LoQ (1 ng/µL) of the assay, and were collected immediately after smoking. These results indicate that samples saliva samples should be collected at least 30 minutes after smoking.

iv. Microbial Interference

A microbial interference study was performed to assess whether microbial DNA affects the performance of the GlutenID Celiac GHR Test. Five DNA samples representing the four tested haplotypes (DQ2, DQ8, DQ7, and DQ2.2) were spiked with and without DNA from the following five microbes:

- Lactobacillus casei
- Staphylococcus epidermidis
- Streptococcus mutans
- Aggregatibacter actinomycetemcomitans
- Candida albicans

All samples were tested in triplicate. The combined results are summarized in the following table:

Haplotypes	No. of Replicates	No. of Correct Calls	No. of Incorrect Calls	No. of FQC	% of FQC	% of Concordance
DQ2.2	15	15	0	0	0%	100%
DQ2.5*	30	30	0	0	0%	100%
DQ7	15	15	0	0	0%	100%
DQ8	15	15	0	0	0%	100%
Total	**75**	**75**	**0**	**0**	**0%**	**100%**
*Includes two heterozygous samples						

No samples failed QC and all replicates produced correct genotype calls in the presence of microbial DNA, demonstrating no interference from the DNA from the tested microbes.

6. Assay Reportable Range:

Not applicable

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

i. Reagent stability:

The GlutenID Celiac GHR Test uses the reagents for NGS to determine an individual's GHR for celiac disease. The NGS sequencing reagents and related commercially available reagents should be stored under recommended conditions and used within the expiration date per the manufacturers' instruction for use.

For PCR, a GlutenID Master Mix containing primers, polymerase and dNTPs are prepared and aliquoted by the lab and stored at -20°C. To determine the stability of the GlutenID Master Mix, three different lots of Master Mix (M1, M2, M3) were prepared from component reagents and stored at -20°C. The stability of the Master Mix was tested at 1, 4, 8, 12, 13 weeks and 6, 12, 18, 24 and 25 months. At each testing time point, three production lots were prepared by including each of three Master Mix lots for PCR and additional critical reagents for sequencing and library preparation (All critical reagents are stored at the recommended condition within their expiration date per the manufacturers' instructions for use). At each designated timepoint, a 6-sample panel with haplotypes DQ2, DQ2+DQ2, DQ8, DQ8+DQ8, DQ2.2 (half-DQ2), DQ7 covering the six celiac genetic risk SNPs was tested in replicates of three for each sample across three lots, yielding a total of 54 results (6 samples x 3 replicates x 3 lots). The stability study is on-going; the already collected data supports the stability of the Master Mix up to 12 months at -20°C.

The stability of the aliquoted Master Mix under freeze/thaw cycles was evaluated. The results indicated that the aliquots of Master Mix are stable up to two freeze/thaw cycles.

ii. Stability of collection device and specimens:

Saliva samples are collected with the ORAcollect·Dx OCD-100. Refer to K152464 for pre-collection shelf-life stability of the collection device, stability of samples post-saliva collection, and freeze-thaw stability of samples stored in this collection device.

iii. Controls

The reagent performance used for the GlutenID Celiac GHR Test is verified daily by running, prior to use, the following quality control materials:

- Negative Control: Non-template control (NTC) with no DNA consisting of sterile water at the same volume as DNA samples and set up as an "unknown" sample in every run.
- Positive Control: Commercially purchased cell line which is positive for homozygous half-DQ2+half-DQ2 (DQ2.2+DQ2.2).

8. Detection Limit:

The Limit of Detection (LoD) of the GlutenID Celiac GHR Test was determined by testing six unique DNA samples representing haplotypes: DQ2, DQ2+DQ2, DQ8, DQ8+DQ8. DQ2.2+DQ7 and DQ2.2+DQ7. Each sample was tested at three DNA concentrations levels:

10 ng/µL, 5 ng/µL and 1 ng/µL, in replicates of two, using three different lots of reagents. The study yielded 36 results (6 samples x 2 replicates x 3 lots) per DNA concentration level and a total of 108 results (36 x 3 DNA levels). LoD was defined as the lowest DNA concentration at which at least 95% of samples resulted correct calls by the GlutenID Celiac GHR Test.

The results showed that, in a total of 108 replicates analyzed, no samples failed QC (FQC) and 100% correct genotype calls were observed for all samples, all reagent lots and all DNA concentration levels. The LoD of the GlutenID Celiac GHR Test is set as 1 ng/µL.

9. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Comparison with Sanger Bi-directional Sequencing:

The accuracy of the GlutenID Celiac GHR Test was assessed by evaluating a panel of samples that are heterozygous and homozygous for the reported haplotypes and covering all the possible results provided by the assay. A total of 143 samples representing all genetic variants tested by the assay were analyzed and compared with bi-directional Sanger sequencing (reference method) to calculate percent agreement with the sequencing results considered to be "truth." The number of samples for each haplotype is based on population frequencies reported in Ojnaga et al., (2018) *Enferm Dig* 110:421−426. These samples included 20 unique samples representing the wild-type genotype (homozygous common) and 123 samples positive for the four celiac risk haplotypes:

- HLA-DQ2.5 (rs2187668) wildtype (CC), heterozygous (CT), homozygous (TT)
- HLA-DQ8 (rs7454108) wildtype (TT), heterozygous (TC), homozygous (CC)
- HLA-DQ7 (rs4639334) wildtype (AA), heterozygous (AG), homozygous (GG)
- HLA-DQ2.2 (rs7775228) wildtype (GG), heterozygous (GT), homozygous (TT)

The percent agreement (PA) for each haplotype, the overall percent agreement and two-sided 95% CIs were calculated. The PAs per haplotype from clinical samples were calculated separately, and the results are summarized in the tables below:

DQ2.5				
Clinical Samples	**Genotype**	**Sanger Bi-directional Sequencing**		
		C/C	C/T	T/T
GlutenID Celiac GHR Test	C/C (homozygous common)	20	0	0
	C/T (heterozygous)	0	20	0
	T/T (homozygous rare)	0	0	20
	FQC	0	0	0
Total=60		**20**	**20**	**20**
PA (C/C | C/C) = 100% (20/20) with 95% CI: 83.9%−100% PA (C/T | C/T) = 100% (20/20) with 95% CI: 83.9%−100% PA (T/T | T/T) = 100% (20/20) with 95% CI: 83.9%−100% Percent of FQC is 0.0% (0/60) 95% CI: 0.0%−6.0%				

DQ8				
Clinical Samples	**Genotype**	**Sanger Bi-directional Sequencing**		
		T/T	T/C	C/C
GlutenID Celiac GHR Test	T/T (homozygous common)	20	0	0
	T/C (heterozygous)	0	20	0
	C/C (homozygous rare)	0	0	3
	FQC	0	0	0
Total=43		**20**	**20**	**3**
PA (T/T | T/T) = 100% (20/20) with 95% CI: 83.9%−100% PA (T/C | T/C) = 100% (20/20) with 95% CI: 83.9%−100% PA (C/C | C/C) = 100% (3/3) with 95% CI: 43.9%−100% Percent of FQC is 0.0% (0/43) with 95% CI: 0.0%−8.2%				

DQ7				
Clinical Samples	**Genotype**	**Sanger Bi-directional Sequencing**		
		A/A	A/G	G/G
GlutenID Celiac GHR Test	A/A (homozygous common)	20	0	0
	A/G (heterozygous)	0	20	0
	G/G (homozygous rare)	0	0	10
	FQC	0	0	0
Total=50		**20**	**20**	**10**
PA (A/A | A/A) = 100% (20/20) with 95% CI: 83.9%−100% PA (A/G | A/G) = 100% (20/20) with 95% CI: 83.9%−100% PA (G/G | G/G) = 100% (10/10) with 95% CI: 72.3%−100% Percent of FQC is 0.0% (0/50) with 95% CI: 0.0%−7.1%				

DQ2.2 (rs7775228)				
Clinical Samples	**Genotype**	**Sanger Bi-directional Sequencing**		
		G/G	G/T	T/T
GlutenID Celiac GHR Test	G/G (homozygous common)	20	0	0
	G/T (heterozygous)	0	20	0
	T/T (homozygous rare)	0	0	10
	FQC	0	0	0
Total=50		**20**	**20**	**10**
PA (G/G | G/G) = 100% (20/20) with 95% CI: 83.9%−100% PA (G/T | G/T) = 100% (20/20) with 95% CI: 83.9%−100% PA (T/T | T/T) = 100% (10/10) with 95% CI: 72.3%−100% Percent of FQC is 0.0% (0/50) with 95% CI: 0.0%−7.1%				

Calculation of PA per allelic variant between the GlutenID Celiac GHR Test results and bi-directional Sanger sequencing was 100% concordant for heterozygous and homozygous

DQ2.5, DQ8, DQ7, DQ2.2 haplotypes. The results showed 100% (203/203) of concordance between GlutenID Celiac GHR test results and the Sanger sequencing method (the 95%CI is 98.1%–100%). The percentage of FQC per allelic variant was also calculated and was 0% (0/203) with 95%CI: (0.0%–1.9%). The study showed that the GlutenID Celiac GHR Test was able to correctly genotype all samples analyzed.

Percentages of variants in the target population as reported by Liu *et al.* is presented in the table below:

Variant	White N (%)	African American N (%)	Asian N (%)	Hispanic, Biracial, & Others N (%)	Total
DQ2+DQ2	250 (1.4)	28 (1.2)	1 (0.2)	80 (0.6)	359 (1.1)
DQ2+DQ8	415 (2.4)	30 (1.8)	4 (0.7)	254 (2.2)	703 (2.2)
^DQ2+half-DQ2	None				
DQ8+DQ8	385 (2.2)	6 (0.3)	5 (0.9)	28 (2.7)	703 (2.2)
^DQ8+half-DQ2	None				
^half-DQ2+half-DQ2					
^DQ7+half-DQ2 DQ2(trans)					
*DQ2.5+DQX DQ2(cis)	3376 (19.2)	472 (20.6)	68 (11.8)	1530 (13.5)	5446 (17.1)
*DQ8+DQX	2803 (15.9)	172 (7.5)	51 (8.9)	2613 (23.1)	5639 (17.8)
^half-DQ2	None				
^DQ7					
Non-celiac genetics (NCG)	10,386 (59.0)	1582 (69.1)	447 (77.6)	6501 (57.6)	18,916 (59.5)
Total	17,615	2290	576	11,285	31,766

Diplotype combinations are represented by the following symbolic nomenclature:
*DQX is any haplotype except DQ2.5, DQ8
^The DQ2(trans) haplotypes (DQ2.2 and DQ7) representing 1.4-5.3% of the general population were not included in the study.
Prevalence of DQ2(trans) is <5% of all patients with celiac disease.

Technical (analytical) positive predictive values for GlutenID Celiac GHR Test results were >92.4%.

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity and Clinical Specificity:

Not applicable

2. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

a. Disease Association and Pathogenesis

The pathophysiology of celiac disease involves a systemic, abnormal immune response to dietary gluten in genetically predisposed individuals. Immune cells attack the small intestine and other tissues causing symptoms such as diarrhea, malabsorption, and anemia.

HLA-DQA1 and HLA-DQB1 genes encode the alpha and beta chains of the HLA-DQ2 and HLA-DQ8 heterodimeric Major Histocompatibility complex (MHC) Class II surface proteins expressed on the surface of antigen presenting cells. MHC Class II proteins on the surface of dendritic cells and monocytes present antigens to immune system T-cells initiating response to foreign antigens. Gluten-reactive T-cells can cause chronic inflammation of the small intestine with accompanying villus atrophy leading to malabsorption. The HLA-DQ2.5 haplotype is present in 95% of patients diagnosed with celiac disease and the HLA- DQ8 haplotype is detected in <5% (Taylor 2015[1]). Rarely, HLA-DQ2 is inherited in the form of two separate haplotypes HLA-DQ7 and HLA-DQ2.2 (one half from each parent) encoding alpha and beta chains differing by only two amino acids from DQ2.5 and conferring similar celiac disease risk (Pisapia 2020[2]). The DQ2.5 haplotype is commonly referred to as DQ2(cis) while the DQ7+DQ2.2 haplotype is called DQ2(trans). Although a hetero or homozygous DQ7 haplotype inherited without DQ2.2 confers only slightly increased risk above non celiac genetics (NCG), laboratory testing for celiac genetic risk must include all four haplotypes (DQ2.5, DQ8, DQ2.2, and DQ7) to identify presence or absence of DQ2(trans).

b. Risk Categorization

Depending on the specific variant combination detected, the GlutenID Celiac Genetic Health Risk Test provides the individuals' genetic health risk for developing celiac disease. Personalized reports are generated for each consumer that help users understand the meaning of their results and any appropriate next steps. The GlutenID Celiac GHR Test uses three categories to define risk. One of these risk categories has been assigned to each genetic result. The risk categorization is based on the reported clinical cases (published references) for each genetic result.

The following three categories are reported for the GlutenID test:

- Increased Risk: Estimated post-test risk is higher than general population risk of 1%.
- Low Risk: Estimated post-test risk is the same or lower than the general population risk of 1%.
- Not likely at Risk: Estimated post-test risk is 0.5% or below.

The GlutenID Celiac Genetic Health Risk (GHR) Test is indicated for detecting the presence or absence of four HLA-DQ haplotype variants associated with the risk of

[1] Taylor et al., (2019). Celiac Disease. GeneReviews [Internet] https://www.ncbi.nlm.nih.gov/books/NBK1727

[2] Pisapia et al., (2020). Differential expression of pre-disposing HLA-DQ2.5 alleles in DR5/DR7 celiac disease patients affects the pathological immune response to gluten. Nature Sci Rep 10:17227 https://doi.org/10.1038/s41598-020-73907-2

developing celiac disease. The GlutenID Celiac GHR Test reports variants associated with the HLA-DQ2.5 haplotype, the HLA-DQ8 haplotype, the HLA-DQ7 haplotype, and the HLA-DQ2.2 haplotype. Inheritance of one or more of these haplotypes is associated with higher risk for development of Celiac disease (Megiorni 2009[3]). The GlutenID test uses SNP genotyping to identify four HLA haplotype variants that confer risk for celiac disease (Monsuur 2008[4]).

Likelihood ratios (LR) for each test result were evaluated based on published literature. The confidence intervals for the LR were calculated using the asymptotic method for the ratios of two independent binomial proportions (see CLSI EP12). For each test result, post-test risk (R) was calculated based on the corresponding LR and pre-test risk π as $R/(1-R) = LR \times \pi/(1-\pi)$, where π is pre-test risk of 1%.

In the clinical study described in F. Megiorni, B. Mora, M. Bonamico, M. Barbato, R. Nenna, G. Maiella, P. Lulli, M. Mazzilli "HLA-DQ and risk gradient for celiac disease" in *Human Immunology* 70 (2009), there were 437 subjects with celiac disease, and 551 subjects without celiac disease. Estimations of the LR for different GlutenID Celiac Genetic Health Risk (GHR) Test results were calculated and presented in the table below:

Test Result	LR	95% CI for LR	Post-Test Risk for Pre-Test Risk of 1%	Risk Category Reported by Test
DQ2+DQ2	9.8	(5.6; 17.1)	8%	Increased
DQ2+half-DQ2				
DQ2+DQ8	13.9	(2.3; 83.5)	6%	Increased
DQ8+half-DQ2	4.1	(1.4; 11.9)	4%	Increased
DQ2+DQ7	2.9	(2.4; 3.5)	3%	Increased
DQ2.2+DQ7 (DQ2 trans)				
DQ2 (cis)				
DQ8+DQ8 DQ8 DQ8+DQ7	1.1	(0.7; 1.8)	1%	Low
Half-DQ2+Half-DQ2 (DQ2.2+DQ2.2)	0.48	(0.29; 0.78)	0.5%	Not Likely at Risk
Half-DQ2(DQ2.2)				
DQ7+DQ7				
DQ7				
Non-celiac genetics (NCF)	0.05	(0.03; 0.08)	0.05%	Not Likely at Risk

Individuals who are homozygous for DQ2(cis) are at highest risk for development of celiac disease (Bajor 2019[5]). Conversely individuals who inherit either DQ7 or DQ2.2

[3] Megiorni, et al., (2009). HLA-DQ and risk gradient for celiac disease. Hum Immunol.70:55-59.
[4] Monsuur et al., (2008). Effective detection of human leukocyte antigen risk alleles in celiac disease using tag single nucleotide polymorphisms. PLoS ONE 3(5): e2270.
[5] Bajor et al., (2019). Classical celiac disease is more frequent with a double dose HLA-DQB1*02: A systematic review with meta-analysis. PLoS ONE 14(2): e0212329.

(but not both) are at lower risk for celiac disease but have 50% chance of transmitting a risk allele to future generations.

Historically, the medical literature has focused primarily on celiac disease genetics in individuals of European ancestry where an estimated 25% of this population carries at least one copy of the HLA-DQ2.5 haplotype (Singh 2018[6]). Although celiac disease is a polygenic disorder, over 90% of patients with celiac disease have at least one copy of the HLA-DQ2.5 haplotype.

3. User Studies

a. Saliva collection kit usability study:

Refer to K212745 for the saliva collection device (ORAcollect·Dx OCD-100) instructions for use and to assess the ability of lay users to provide samples adequate for testing.

b. GlutenID Celiac GHR Test user comprehension study:

Objectives:
The user comprehension study was performed to assess user comprehension of the GlutenID Celiac GHR Test reports. The study was performed with a demographically diverse group of study participants 18 years old or older by asking questions for five comprehension categories (purpose of test, limitations, relevant ethnicities, meaning of results, other factors that may have an impact on test results, and appropriate action) of the GlutenID Celiac GHR Test reports.

Methods:
Quota-based sampling per U.S. Census was employed to recruit a diverse set of study participants based on their demographic variables in age, gender, race/ethnicity, education level and geographic region. Study participants who enrolled into the user comprehension study and passed the screening questionnaire to ensure that they met the inclusion criteria for the study were randomly assigned into one of three risk category groups ('Not likely at risk', 'Low risk', and 'Increased risk'). Exclusion criteria were applied in the recruitment stage, as well as during the study data collection. All exclusions were counted and documented during the study and were reported with a clear description of each excluded participants' demographics and detailed reason for exclusion. During the analysis, the demographics of all participants (defined as those who consent to participate) was compared to the demographics of those remaining after exclusion, to evaluate whether the exclusion process potentially introduced any bias.

Of the 15 total possible GHR Reports that can be generated by the GlutenID Celiac GHR Test, three were selected for the User Comprehension Study. The selected Reports summarized in the table below cover all the possibilities for number of variants and risk

[6] Singh et al., (2018). Global prevalence of celiac disease: systematic review and meta-analysis. Clin Gastroenterol and Hepatol. 16:823-83.

categories associated with celiac disease that can be reported by the GlutenID Celiac GHR Test:

- *Number of haplotypes detected*: a total of three different reports can be obtained: 0, 1, or 2 haplotypes detected.

- *Risk category:* three different risk categories ('Not likely at risk', 'Low risk', and 'Increased risk') can be provided by the test, which represent the risk for developing celiac disease.

	GHR Report	Number of Haplotypes Detected	Risk Category for Celiac Disease
1	GlutenID NCG*	0	Not likely at risk
2	GlutenID DQ2cis	1	Low risk
3	GlutenID DQ2+DQ8	2	Increased risk

*Non-Celiac Genetic

As the GHR Report for 'Variant not determined' was not included in the User Comprehension Study, the 'No Results' email text that the user will receive when the laboratory is unable to complete DNA testing due to insufficient or no human DNA being present in the collected saliva sample was also shown to all study participants along with two questions designed to evaluate whether the key concepts are communicated effectively. Each GHR Report includes the following sections: 'Your Result' (Result Report), 'Scientific Details', 'Frequently Asked Questions', and 'Glossary of Terms'

User comprehension was tested through a two-step process. First, participants' comprehension was tested prior to viewing the Educational Module, and GHR Reports. The Pre-Test questionnaire included basic demographics (for inclusion/ exclusion criteria) and baseline comprehension questions that covered the following comprehension domains: test purpose [PURPOSE], test limitations [LIMITATIONS], race/ethnicity [ETHNICITY], and other factors that may have an impact on the test results [OTHER FACTORS]. Second, on completion of reviewing a representative sample of all the material that is included in the Education Module and the GlutenID reports, participants were provided with the Post-Test questionnaire to complete (approximately 20 minutes). The Post-Test questionnaire included comprehension questions designed to measure all six comprehension domains: test results [RESULTS] and appropriate action [NEXT STEPS] in addition to PURPOSE, LIMITATIONS, ETHNICITY, OTHER FACTORS. The RESULTS and NEXT STEPS domains were only included in the Post-Test questionnaire because they represented specific results that participants could not know prior to viewing the GHR reports and related materials. Each comprehension domain included at least two comprehension questions.

The GHR Reports and Supplemental Materials were reviewed by a Certified Genetic Counselor (GC) to ensure that the materials sufficiently cover the relevant general and variant-specific concepts [comprehension domains]. Prior to recruitment for the user comprehension study, the "No Result" email and test report materials (GHR Report, Education Module, and Supplemental Materials), with content targeted no more difficult than 8th grade reading level, were evaluated for readability.

A total of 361 participants were engaged in this study. Of the 361 participants, 18 were determined to be ineligible and qualified as exclusions, leaving 343 eligible participants in the study. Of those eligible, a total of 3 failed to complete the Pre-Test. A total of 340 completed the Pre-Test and were invited to participate in the POST survey. A total of 26 failed to complete the Post-Test, leaving 314 complete eligible participants at study completion. This means the study had a completion rate of 92%. There were no statistical differences observed between those who consented (n=361) and those who eventually participated and completed the study (post-exclusion; n=314).

The study was set up to randomly assign each eligible study participant to one of three study arms identified by the three pre-selected test report types, with the following number of eligible participants that successfully completed the User Comprehension Study as summarized in the table below for each study arm.

Study Arm	GHR Report	Total Participants Completed Study
1	GlutenID NCG*	109
2	GlutenID DQ2(cis)	101
3	GlutenID DQ2+DQ8	105
Total		**314**

*Non-Celiac Genetic

Results

Comprehension scores were calculated using the total number of responses received that were correct over the total number of eligible responses received. The calculated proportion for each domain serves as the comprehension score for that domain. The target score for each comprehension domain was 90% across all reports. A mean comprehension rate was calculated across all comprehension question domains and all reports. To evaluate whether the comprehension scores had significantly improved statistically, the pre- and post-test scores in each domain, and overall, were compared by using paired sample t-test. Results summarized in the table below show that each comprehension domain achieved a minimum of 79.3% or higher user comprehension score in the pre-test questionnaire, and 94.0% or higher user comprehension score in the post-test questionnaire, across all reports.

User Comprehension Pre- to Post-Test Scores for all GHR Reports by Domain				
Domain Name	**N**	**Pre-Test (%)**	**Post-Test (%)**	**Improvement (%)**
Education Module	314	88.2	97.8	9.6
Purpose	314	95.1	97.7	2.6
Other Factors	314	94.8	96.7	1.9
Limitations	314	80.2	95.9	15.7
Race/Ethnicity	314	79.3	96.8	17.5
Average of all domains	**314**	**87.5**	**97.0**	**9.5**

User Comprehension Pre- to Post-Test Scores for all GHR Reports by Domain				
Domain Name	**N**	**Pre-Test (%)**	**Post-Test (%)**	**Improvement (%)**
Results	314	N/A	94.4	N/A
Next Steps	314	N/A	96.1	N/A
Variant not determined^	314	N/A	99.5	N/A

^ The lab is unable to complete the DNA tests due to insufficient or no human DNA being present in the collected sample

The overall comprehension scores were of 87.5% and 97.0% across all comprehension domains (i.e., Education Module, Purpose, Other Factors, Limitations and Race/Ethnicity) for the pre-test and post-test questionnaire, respectively. Results of the pre- to post-test comparison by paired sample t-tests showed a statistically significant improvement in the user comprehension scores between the pre-and post-test questionnaire for the Education Module, and the domains of 'Limitations', and 'Ethnicity'. Statistical significance demonstrates that the increase in the user comprehension score was a result of the education material. The 'Purpose" and 'Other Factors' domains showed no significant difference.

When analyzed individually for each of the three GHR reports, the post-test comprehension scores for each domain are 91% or higher (Next Steps domain in the NCG (GlutenID Non-Celiac Genetic report has a post-test score of 91%) as shown in the table below.

Post-Test Comprehension Rates (%) by GHR Report Type			
Domain Name	**GlutenID NCG***	**GlutenID DQ2(cis)**	**GlutenID DQ2+DQ8**
Education	97	99	97
Purpose	97	98	98
Other Factors	97	97	97
Limitations	94	95	99
Ethnicity	97	97	97
Results	97	94	97
Next Steps	91	94	98
Average of all Domains	**96**	**96**	**98**
Variant not determined^	99	100	100

* Non-Celiac Genetic

^ The lab is unable to complete the DNA tests due to insufficient or no human DNA being present in the collected sample

The following table summarizes the user comprehension scores (post-test only) broken out by demographic category (age, sex, race/ethnicity, education level, and geographic region) per comprehension domain for the overall study (all three GHR reports combined). Every demographic category scored 92% or higher in every comprehension domain across all report types. Results showed that participant demographics were unrelated to user comprehension.

User Comprehension Post-Test Scores for all Reports by Demographic									
		Comprehension Domain							
		Education	Purpose	Other Factors	Limitations	Ethnicity	Next Steps	Results	Variant not determined
	N	Mean (%)							
Overall	314	98	98	97	96	97	96	94	100
Age									
18-34	101	98	98	99	95	97	96	95	100
35-49	112	98	99	97	96	98	97	96	100
50+	101	97	96	94	96	96	96	92	99
Sex^									
Male	161	97	98	96	96	96	95	94	100
Female	152	99	98	97	96	97	97	95	99
Race/Ethnicity*									
White Alone	184	99	98	98	96	97	97	94	99
Non-White	130	97	97	96	95	96	95	95	100
Education**									
Some College	152	98	97	96	96	97	97	95	99
Bachelor's Degree or Higer	162	97	98	97	96	97	95	94	100
Geographic Region									
Northeast	69	97	97	97	98	97	96	94	99
Midwest	67	97	96	97	98	97	96	92	99
South	74	98	97	96	94	97	95	96	100
West	104	99	99	97	98	97	95	96	100

^ one participant identified as non-binary or other "not listed" gender

* Non-White: include African American, Asian American, Middle Eastern/North African, Native American,Native Hawaiian/Other Pacific Islander, biracial or Multiracial, and Hispanic

** Some college: include associate degree or less

c. Frequently Asked Questions (FAQ) Material:

A Frequently Asked Questions (FAQs) section was developed and included in the GlutenID Celiac GHR Test report. The FAQs section provides users with information to adequately understand the purpose, limitations, and meaning of results of the test and was developed to be consistent with the manufacturer's labeling design, identification of communication messages, and label comprehension. The concepts covered in the FAQs include: test results, meaning of test results, purpose of the test, test limitations, other risk factors that contribute to disease, relevance of race and ethnicity on test results, how the test results may affect the user's family and children, appropriate follow-up procedures, links to resources for additional information.

The questions included in the FAQ section for each of the test report included the following:

- What is celiac disease?
- Can celiac disease affect anyone?

- How is celiac disease treated?
- What is the purpose of the GlutenID test?
- How accurate is the GlutenID test?
- Does the GlutenID test look at my whole genetic profile?
- My report says I have NONE of the celiac risk variants, what does this mean?
- My result says, 'Not Likely at Risk', what next steps should I take?
- Based on my results, should I immediately remove gluten from my diet?
- What are the limitations of the GlutenID test?
- How does this result affect my family?
- How does a person's ethnic background impact the results?
- If I am not of European descent does the test apply to me?
- Where can I find more information?
- How do I get an appointment with a genetic counselor?

Each GlutenID Celiac Genetic Health Report provides information to the FAQs that are specific to celiac disease risk haplotypes.

d. User Opt-In page:

Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Not applicable

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.