510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K182784

B. Purpose for Submission:

New device

C. Measurand:

Two variants (Y179C and G396D) in MUTYH gene

D. Type of Test:

Qualitiative genetic test for detection of two variants in MUTYH gene

E. Applicant:

23andMe, Inc.

F. Proprietary and Established Names:

23andMe Personal Genome Service (PGS) Risk Report for MUTYH-Associated Polyposis (MAP)

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.6090

2. Classification:
   Class II

3. Product code:
   QAZ

4. Panel:
   Pathology

H. Intended Use:

1. Indication(s) for use:
   The 23andMe Personal Genome Service (PGS) uses qualitative genotyping to detect select clinically relevant variants in genomic DNA isolated from human saliva collected from individuals ≥18 years with the Oragene Dx model OGD500.001 for the purpose of reporting and interpreting genetic health risks, including the 23andMe PGS Genetic Health Risk Report for MUTYHAssociated Polyposis. The 23andMe PGS Genetic Health Risk Report for MUTYHAssociated Polyposis is indicated for reporting of the Y179C and the G396D variants in the MUTYH gene. The report describes if a person is at increased risk of developing colorectal cancer. The two variants included in this report are most common and best studied in people of Northern European descent and may not represent the majority of the MUTYH variants in people of other ethnicities. The test report does not describe a person's overall risk of developing any type of cancer, and the absence of a variant tested does not rule out the presence of other variants that may be cancer-related. This test is not a substitute for visits to a healthcare provider for recommended screenings or appropriate follow-up and should not be used to determine any treatments.

2. Special conditions for use statement(s):
   a. For over-the-counter (OTC) use.
   b. The test does not diagnose cancer or any other health condition and should not be used to make medical decisions. Results should be confirmed in a clinical setting before taking any medical action.
   c. This test is not a substitute for visits to a healthcare provider for recommended screening or appropriate follow-up. It is recommended that users consult with a healthcare provider if there are any questions or concerns about the test results or their current state of health.
   d. The 23andMe PGS Genetic Health Risk Report for MUTYH-Associated Polyposis (MAP) detects only two variants in MUTYH gene and does not detect all genetic variants in this gene associated with increased risk of developing colorectal cancer (CRC). There are more than 100 variants in the MUTYH gene known to be associated with increased risk of developing cancer. The absence of a variant tested does not rule out the presence of other genetic variants that may be disease-related.
   e. The test is intended for users ≥ 18 years old.
   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 6-33%.
   g. Three potentially interfering mutations near Y179C, and four potentially interfering mutations near G396D that are within the binding region for the variant being tested have been identified and are noted below. Interference due to these mutations was not tested.

MYTUH variant	**Potentially Interfering Mutation**
Y179C	rs190500741, rs533899702, rs201678305
G396D	rs559963863, rs529008617, rs3219490, rs531232542

   h. A user's race, ethnicity, age, and sex may affect how the genetic test results are interpreted.

i. It is important for the user to discuss their personal or family history of cancer with a healthcare professional. If the user has a personal or family history of cancer, or think they may have symptoms of cancer; the user should consult with their healthcare provider about appropriate testing.
j. Subject to meeting the limitations contained in the special controls under regulation 21 CFR 866.6090.

4. Special instrument requirements:

   Tecan Evo, Illumina iScan and GenomeStudio system (qualified by 23andMe)

I. Device Description:

The 23andMe Personal Genome Service (PGS) is an over-the-counter (direct-to-consumer), qualitative in vitro diagnostic DNA test that provides genotype information using a customer's DNA.

Customer saliva is self-collected using the Oragene·Dx® Device manufactured by DNA Genotek, Inc. (previously cleared for carrier screening indications under K141410, which consists of a sealable collection tube containing a stabilizing buffer solution. Sample are shipped to one of two laboratories for testing.

DNA is isolated from the saliva and tested in a multiplex assay using a customized genotyping beadchip, reagents and instrumentation manufactured by Illumina and qualified by 23andMe.

The raw data is generated using Illumina GenomeStudio software and analyzed using the 23andMe's proprietary Coregen software, where a genotype is determined for each tested SNP. The results are used to generate reports for the customer that provide information about the detected genotypes.

Customer reports for the MUTYH variants include variant(s) detected and associated risk of cancer as reported in literature and guidelines. If no variant was detected, that information is also provided. The reports are designed to present scientific concepts to users in an easy-to-understand format, as well as the limitations of the testing and test results.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   23andMe Personal Genome Service (PGS) Risk Report for BRCA1/BRCA2 (Selected Variants)

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

   DEN170046

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Technology	Customized Illumina BeadChip	Same platform
Specimen type	Saliva	Same
Collection Device	Oragene·Dx® saliva collection device (OGD-500.001)	Same
Measurand	DNA	Same
Instruments and software	Tecan Evo, Illumina iScan and Genome Studio Coregen	Same

Differences		
Item	Device	Predicate
Variants to be reported	Y179C and G396D in MUTYH gene	185delAG and 5382insC in BRCA1 and 6174delT in BRCA 2 gene
Indication	Qualitative reporting of risk for MUTYH-Associated Polyposis (MAP) and Colon Cancer	Qualitative reporting of risk for Breast Cancer

K. Standard/Guidance Document Referenced (if applicable):

Not applicable

L. Test Principle:

The assay uses multiplex microarray technology for the simultaneous detection of variants in human DNA. The BeadChip v5 assay (Illumina Infinium HumanOmniExpress-24 format chip) consists of silicon wafers etched to form wells loaded with silica beads, on which oligonucleotide capture probes are immobilized. DNA from saliva is fragmented and captured on a bead array by hybridization to immobilized SNP-specific primers, followed by extension with hapten-labeled nucleotides. The primers hybridize adjacent to the SNPs 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 and Illumina iScan instruments are used for extraction and processing of the DNA, and the BeadChip for scanning and quantification of the results. The genotype content is separated, analyzed, and then integrated into pre-defined report templates specific for each condition associated with each genotype. Genotypes are determined using the GenomeStudio and Coregen software packages. For the 23andMe PGS Genetic Health Risk Report for MUTYH-Associated Polyposis (MAP), information on two specific variants (Y179C, c.536A>G in exon 7 and G396D, c.1187G>A in exon 13) in the MUTYH gene are integrated into the report.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility studies were conducted for the two variants reported in MUTYH gene. The reproducibility studies were designed to determine the imprecision due to assay run, lot, instrument, operator, day and site. DNA samples were procured and genotyped in blinded fashion. Genotypes of the DNA samples were confirmed through bidirectional Sanger sequencing. The study included one unique specimen for each genotype of the marker (i.e., specimens representing wild-type, heterozygous and homozygous cases for each allele).. Each sample was run in triplicates and genotyped at the two laboratory sites on three days using three laboratory operator teams at each site, three lots of reagents (chosen at random from all available), three Tecan instruments, and three iScan instruments. Total number of replicates per sample is 162 except Y179C. Due to the rarity of Y179C marker, the "CC" genotype was tested with 2 instruments (Tecan and iScan), over 3 days, at each of 2 laboratory sites (N=36).

Among samples with valid calls, the precision study yielded 100% correct genotype calls including samples that were rerun. Information regarding samples that failed quality control (FQC) was also evaluated (as listed in Table 1). The data presented below are based on FQCs following a single run. Samples with FQC on the first run are re-tested in accordance with the laboratory protocol (i.e., one repeat test following invalid result). The results are shown in Table 1

Tables 1A-B. Precision Study Results Stratified by Site and Genotype

Table 1A. MUTYH Y179C (i5012768) Results

Genotype	Number of Replicates (including FQCs)	Number of Correct Calls	Number of Incorrect Calls	Number of FQCs	Percentage of FQCs
Site 1					
Homozygous Common	81	81	0	0	0%
Heterozygous	81	81	0	0	0%
Homozygous Rare	18	18	0	0	0%
Total	180	180	0	0	0%
Site 2					
Homozygous Common	81	80	0	1	0%
Heterozygous	81	73	0	8	10%
Homozygous Rare	18	12	0	6	33%
Total	180	165	0	15	8%

Table 1B. MUTYH G396D (rs36053993) Results

Genotype	Number of Replicates (including FQCs)	Number of Correct Calls	Number of Incorrect Calls	Number of FQCs	Percentage of FQCs
Site 1					
Homozygous Common	81	81	0	0	0%
Heterozygous	81	81	0	0	0%
Homozygous Rare	81	81	0	0	0%
Total	243	243	0	0	0%
Site 2					
Homozygous Common	81	80	0	1	0%
Heterozygous	81	75	0	6	7%
Homozygous Rare	81	73	0	8	10%
Total	243	228	0	15	6%

b. Linearity/assay reportable range:

Not applicable.

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

The assay requires two types of controls: the sample processing control and the reproducibility control. The information provided demonstrates that the sample processing control is stable for up to three months and the reproducibility control is stable for up to 12 months. See DEN140044 for detailed information.

d. Detection limit:

The Limit of Detection (LoD) study was performed to determine the lowest concentration of DNA that is necessary for successful assignment of the correct Y179C and G396D variants using the 23andMe PGS test. Study samples were obtained from an external vendor and the 23andMe biobank based on their listed genotypes and included both homozygous and heterozygous common genotypes for each variant. Each sample, including three replicates per sample, was diluted to three different DNA concentrations (5, 15, and 50 ng/µl) and genotyped by the PGS test in a blinded fashion using 3 lots of reagents. To confirm the genotype call, each sample was sequenced by bidirectional Sanger sequencing. Genotype calls from the test were compared with genotypes from Sanger sequencing to determine the rates of correct genotype calls at each DNA concentration.

The LoD was defined as the lowest DNA concentration at which at least 95% of samples yielded the correct call. This study yielded 100% correct calls per genotype for all samples across all reagent lots, at all sample concentrations tested. Therefore, the study passed the acceptance criteria of 95% correct calls at the lowest

concentration tested (5 ng/µL). The performance requirement for the PGS Test, specified in the laboratory SOPs, is set at a minimum of 15 ng/µL DNA and maximum of 50 ng/µL DNA.

e. Analytical specificity:

Interfering Substances – Endogenous and Exogenous Substances

A series of studies were conducted to assess the effects of endogenous substances, exogenous substances, microbial substances, and smoking on the 23andMe PGS Test. The results of the Endogenous and Exogenous Interference studies can be found in the Decision Summary for DEN140044. No interference was observed with the endogenous substances tested. The study indicated that saliva samples should be collected at least 30 minutes after eating, drinking, chewing gum, using mouthwash.

Interfering Mutations

Analyses were performed to identify potentially interfering variants within the 50-nucleotide probe-binding regions of the two MUTYH variants detected by the test. Three potentially interfering mutations near Y179C, and four potentially interfering mutations near G396D that are within the binding region for the variant being tested have been identified (see list in Table 2). The specific mutations potentially interfering with detection of each tested variant are noted below. Interference due to these mutations was not tested. Therefore it is listed in the limitation statements in the package insert.

Table 2 Potentially Interfering Mutations in MUTYH gene

MYTUH variant	**Potentially Interfering Mutation**
Y179C	rs190500741, rs533899702, rs201678305
G396D	rs559963863, rs529008617, rs3219490, rs531232542

Smoking and Microbial Interference

The effects of smoking before the saliva collection and microbial interference were performed. The studies indicated that saliva samples should be collected at least 30 minnutes after smoking and there is no effect on the accuracy of the test by five microbes that maybe found in human saliva. See DEN140044 for additional information.

f. Assay cut-off:

Not applicable.

g. Specimen Stability

Saliva samples for testing are collected with the Oragene·Dx collection device. The claimed specimen stability is 12 months at ambient temperature. See K110701 for sample stability information.

f. Shipping Stability:

Saliva samples are shipped for testing in the Oragene·Dx collection device. Environmental conditions experienced during shipping were simulated by subjecting samples to freeze-thaw cycles (samples stored at high temperatures that could be experienced during shipping were evaluated in specimen stability. The claimed shipping stability is up to three freeze-thaw cycles. See K110701 for sample shipping stability information.

2. Comparison studies:

a. Method comparison with Sanger Bidirectional Sequencing:
Accuracy was evaluated through calculation of agreement of the genetic variant determinations between the 23andMe PGS test MUTYH results and Sanger bidirectional sequencing (comparator) results. All Sanger bidirectional sequencing was performed at an independent laboratory site. Saliva samples were randomly selected from the 23andMe customer biobank based on predetermined genotypes and the minimum volume required for testing. All chosen samples were then genotyped using Sanger bidirectional sequencing. Genotyping results were compared between the PGS test and bidirectional sequencing to calculate percent agreements with the sequencing results used as the reference. The comparison study results for the two variants are shown in Table 3 below. The accuracy data generated for each test report met the Manufacturer's pre-defined acceptance criteria: a minimum of 99% positive percent agreement (PPA) and negative percent agreement (NPA) for each genotype.

Table 3. Percent Agreement for MUTYH Variants by Genotypes

Genotype by Sanger	PGS Test Genotype Call						%PPA	%NPA	95% CI‡
	Correct*	Incorrect*	No Call	FQC	FQC %	Total Sample #			
MUTYH Y179C Homozygous Common	25	0	0	0	0%	25	100	100	86.3-100
MUTYH Y179C Heterozygous	26	0	0	0	0%	26	100	100	86.8-100
MUTYH Y179C Homozygous Rare	14	0	0	1	7%	15	100	100	76.8-100
MUTYH G396D Homozygous Common	26	0	0	0	0%	26	100	100	86.8-100
MUTYH	27	0	0	0	0%	27	100	100	87.2-100

G396D Heterozygous									
MUTYH G396D Homozygous Rare	25	0	0	0	0%	25	100	100	86.3-100

*Relative to Sanger sequencing
‡Clopper-Pearson exact method

b. Matrix comparison:

Not applicable. This test is for use with human saliva samples only.

3. Clinical studies:

a. Disease Description and Clinical Summary:

The MUTYH gene encodes for an enzyme called MYH glycosylase, which is involved in the repair of DNA. This enzyme corrects particular errors that are made when DNA is copied (DNA replication) in preparation for cell division. During normal cellular activities, guanine sometimes becomes altered by oxygen, which causes it to pair with adenine instead of cytosine. MYH glycosylase fixes this error so mutations do not accumulate in the DNA and lead to tumor formation. This type of repair is known as base excision repair and can lead to a type of cancer known as MUTYH-associated polyposis.

MUTYH-associated polyposis is considered to be autosomal recessive polyposis syndrome (one variant must be inherited by each parent), though monoallelic carriers may have slightly increased risk for colorectal cancer. Affected patients have multiple colorectal adenomas and an increased risk of colorectal cancer.

The two most common MUTYH gene pathogenic variants in Western Europeans and North Americans are Y179C (rs34612342, c.536A>G in exon 7) and G396D (rs36053993, c.1187G>A in exon 13) (Nielsen 2012; ClinVar). (previously referred to as Y165C and G382D, respectively). However, pathogenic variants at different loci have been reported in other populations . Patients with MUTYH-associated polyposis may be homozygous or compound heterozygous for these or other pathogenic variants in the MUTYH gene.

These two variants cover about 80-90% of all MUTYH pathogenic variants in northern European populations (GeneReviews, Nielsen 2012; Win 2014; Cleary 2009). The MUTYH carrier frequencies in this population are about 1-2%, from which a prevalence of 1:10,000 to 1:40,000 could be calculated (GeneReviews, Nielsen 2012). These two variants have also been observed in people of other ethnicities.

Table 4 below listed the frequency of MUTYH variants in 23andme customors and public databases.

Table 4 Frequency of MUTYH variants in 23andMe customers

Variant Name	European	African American	Ashkenazi Jewish	East Asian	Hispanic or Latino	South Asian
Y179C	0.42%	0.12%	0.00%	0.00%	0.29%	0.05%
G396D	1.16%	0.41%	0.00%	0.01%	1.02%	0.05%

Clinical validity of the variants is supported by published data and NCCN and ACG guidelines. Clinical data relating to pathogenic variants in MUTYH is summarized in Table 5 below.

Table 5 Health Risk Estimates and Test Interpretation for Colorectal Cancer

Genotype	Risk Estimates	References
0 variant	4.2%	SEER Cancer Statistics Review, 1975-2015[1]
1 MUTYH variant	Uncertain to slightly increase risk	NCCN 2017[2]; Win 2014[3]
2 MUTYH variants	43-100%	ACG Guidelines[4], Nielsen 2012[5]

Individuals with *MUTYH*-associated polyposis usually develop between 10 to 100 colorectal polyps by the fifth or sixth decade. Individuals with *MUTYH*-associated polyposis are at high risk for developing CRC, and approximately 60 percent of patients have CRC at presentation. In contrast, the risk of CRC in monoallelic *MUTYH* carriers appears to be only marginally increased with an estimated lifetime risk of slightly evelvated[2,3].

b. Other clinical supportive data:

i. User Comprehension Study

User comprehension studies were performed to assess the comprehension of the Genetic Health Risk report. See DEN160026 supportive user comprehension studies.

[1] Noone AM, Howlader N, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2015, National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/csr/1975_2015/, based on November 2017, SEER data submission.
[2] National Comprehensive Cancer Network. Genetic/Familial High-Risk Assessment: Colorectal (Version 3.2017).
[3] Win AK et al. Risk of colorectal cancer for carriers of mutations in MUTYH, with and without a family history of cancer. Gastroenterology. 2014, 146(5):1208-11.e1-5.
[4] American College of Gastroenterology (ACG) clinical Guideline: Genetic testing and management of hereditary gastrointestinal cancer syndromes. Syngal S et al. Am J Gastroenterol. 2015, 110(2):223-62
[5] Nielsen M et al. MUTYH-Associated Polyposis. GeneReviews, 2012

ii. Frequently Asked Questions Material

The Manufacturer has developed a Frequently Asked Questions (FAQ) section for the MUTYH-Associated Polyposis (MAP) Genetic Health Risk (GHR) report, which is included in the test report and accessible to the user on the Manufacturer's public website. The FAQs are specific to the variants and disease risk associations being reported, where applicable. The FAQ section was created to provide users with information to adequately understand the purpose, limitations and meaning of the results of the test. The FAQ section was developed using methodology consistent with the Manufacturer's labeling design, identification of primary communication messages, and label comprehension. The concepts covered in the FAQ section include: the test results, purpose of the test, limitations of the test, relevance of race and ethnicity on test results, meaning of the result, other risk factors that contribute to disease, appropriate follow-up procedures, how the results of the test may affect the user's family and children, and links to resources that provide additional information. Additionally, the FAQ section provides definitions for terminology found in Genetic Health Risk Reports that is used to describe risks associated with detected variants.

iii. User Opt-In Page

Prior to receiving the test results, a pre-purchase page informs users that there is a choice of whether or not to receive the MUTYH-Associated Polyposis (MAP) test report. Users have an opportunity to opt into receiving these results after reviewing important information included in an opt-in page. The opt-in page is provided for the MUTYH-Associated Polyposis (MAP) GHR report users due to the nature of the diseases and associated risks for this report and the fact that this test is not designed to inform clinical decision-making. Users will be directed to a page entitled, "Choose your health reports" which provides the option to exclude this report from the users account. The report selection page includes important information to allow the users to make an informed decision. Results of the MUTYH-Associated Polyposis (MAP) report are locked by default, and will never be shown to users unless they have specifically chosen to receive the report at any time, including after results for other reports have been received.

4. Expected values/Reference range:

Not applicable.

N. Instrument Name:

Illumina iScan BeadChip scanner with GenomeStudio software (qualified by the laboratory)

O. System Descriptions:

1. Modes of Operation:

Same as referenced in DEN140044

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:

   Same as referenced in DEN140044

   Revision Level History:

   A software revision history record for the 23andMe software system software was acceptable.

   Unresolved Anomalies:

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

   EMC Testing:

   Not applicable.

3. Specimen Identification:

   Same as referenced in DEN140044

4. Specimen Sampling and Handling:

   Same as referenced in DEN140044

5. Calibration:

   Same as referenced in DEN140044

6. Quality Control:

   Same as referenced in DEN140044

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The

“Performance Characteristics” Section above:

Refer to K141410 for saliva collection device details and study results.

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type.

R. Conclusion:

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