Ambry Genetics®
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SAMPLE REPORT

Ordered By	Contact ID:405956	Org ID:249	Patient Name:	**Last, First**	
Medical Professional:	Sample Doctor, A		Accession #:	**00-332049**	Specimen #: 44-55-66
Client:	Sample Organization (00403)		AP2 Order #:	205725	Specimen: Blood EDTA (Purple top)
Additional Authorized Recipient:			Birthdate:	01/01/1980	Sex at Birth: F
Sample Genetic Counselor MS, CGC			MRN #:	####	Collected: 05/18/2018
			Indication:	Diagnostic/Family History	Received: 05/19/2018

BRCANext-Expanded™: Analyses of 23 Genes Associated with Hereditary Breast & Gynecologic Cancer

RESULTS

Pathogenic Mutation(s): None Detected
Variant(s) of Unknown Significance: None Detected
Gross Deletion(s)/Duplication(s): None Detected

SUMMARY

NEGATIVE: No Clinically Significant Variants Detected

INTERPRETATION

- No pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected.
- **Risk Estimate:** low likelihood of variants in the genes analyzed contributing to this individual's clinical history.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

Genes Analyzed (23 total): ***ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, DICER1, MLH1, MSH2, MSH6, NBN, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, RECQL, SMARCA4, STK11*** **and** ***TP53*** **(sequencing and deletion/duplication);** ***EPCAM*** **(deletion/duplication only)**.

Order Summary: The following products were included in the test order for this individual. Please note: tests on hold and those that have been cancelled (including reflex testing steps cancelled due to a positive result in a preceding test) are excluded. For additional information, please contact Ambry Genetics.

- BRCANext-Expanded™ (Product Code 8860)

ASSAY INFORMATION

General Information: Ovarian cancer (OMIM #167000) is the fifth most common cancer among women, and it arises in the egg-producing ovaries. It is the leading cause of death from gynecologic malignancy, characterized by advanced presentation with regional dissemination in the peritoneal cavity. Epithelial ovarian cancer is the most common form and arises as a result of genetic alterations sustained by the ovarian surface epithelium. Breast cancer (OMIM #604370 and #612555) is a complex, multifactorial disease in which cells in the breast become abnormal and multiply without control to form malignant tumors. It is estimated that approximately 230,000 females and 2,200 males are newly diagnosed each year and that 1 in 8 women will be diagnosed with breast cancer over the course of a lifetime. The most common form of breast cancer is ductal cancer, which begins in cells lining the ducts that carry milk to the nipple. Other forms of breast cancer begin in the glands that produce milk (lobular cancer) or in other parts of the breast. Breast cancer is the most common malignancy among women in developed countries and family history remains the strongest single predictor of breast cancer risk. Other risk factors for breast cancers include genetic variations, age, gender, alcohol abuse and radiation. Hereditary breast cancers tend to occur earlier in life than non-inherited sporadic cases and are more likely to involve both breasts. Hereditary breast and ovarian cancers caused by mutations in the highly penetrant genes, *BRCA1* and *BRCA2*, appear to be responsible for about 10% of total breast cancers and ovarian cancers. In addition, mutations in other genes are known to contribute to the incidence of breast and ovarian cancers. These other susceptibility genes include *ATM, BARD1, BRIP1, CDH1, CHEK2, DICER1, EPCAM, MLH1, MSH2, MSH6, NBN, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, RECQL, SMARCA4, STK11, TP53*.

Methodology: The **BRCANext- *Expanded*™** test is a comprehensive screen of 23 genes associated with breast and/or gynecologic cancers. Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using standardized methodology and quantified. Sequence enrichment of the targeted coding exons and adjacent intronic nucleotides is carried out by a bait-capture methodology using long biotinylated oligonucleotide probes followed by polymerase chain reaction (PCR) and Next-Generation sequencing. Additional Sanger sequencing is performed for any regions missing or with insufficient read depth coverage for reliable heterozygous variant detection. Variants in regions complicated by pseudogene interference, variant calls not satisfying depth of coverage and variant allele frequency quality thresholds, and potentially homozygous variants are verified by Sanger sequencing. For *BRCA2* and *MSH2*, the Portuguese founder mutation, c.156_157insAlu (also known as 384insAlu), and the coding exons 1-7 inversion, respectively, are detected by next generation sequencing and confirmed by multiplex ligation-dependent probe amplification (MLPA) or PCR and agarose gel electrophoresis. Gross deletion/duplication analysis for the genes sequenced (excluding *PMS2*) is performed using a custom pipeline based on read-depth from NGS data and/or targeted chromosomal microarray with confirmatory MLPA when applicable. Gross deletion/duplication analysis of *PMS2* is performed using MLPA kit P008-B1. If a deletion is detected in exons 13, 14, or 15 of *PMS2*, double stranded sequencing of the appropriate exon(s) of the pseudogene *PMS2CL* will be performed to determine if the deletion is located in the *PMS2* gene or pseudogene. Sequence analysis is based on the following NCBI reference sequences: *ATM*- NM_000051.3, *BARD1*- NM_000465.2, *BRCA1*- NM_007294.3, *BRCA2*- NM_000059.3, *BRIP1*- NM_032043.2, *CDH1*- NM_004360.3, *CHEK2*- NM_007194.3, *DICER1*-NM_177438.2, *MLH1*- NM_000249.3, *MSH2*- NM_000251.1, *MSH6*- NM_000179.2, *NBN*- NM_002485.4, *NF1*- NM_000267.3, *PALB2*- NM_024675.3, *PMS2*- NM_000535.5, *PTEN*- NM_000314.4, *RAD51C*- NM_058216.1, *RAD51D*- NM_002878.3, *RECQL*- NM_002907.3, *SMARCA4*- NM_001128849.1, *STK11*- NM_000455.4, *TP53*- NM_000546.4.

Analytical Range: The **BRCANext- *Expanded*™** test targets detection of DNA sequence mutations in 22 genes (*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, DICER1, MLH1, MSH2, MSH6, NBN, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, RECQL, SMARCA4*, *STK11* and *TP53)* by either Next-Generation or Sanger sequencing of all coding domains and well into the flanking 5' and 3' ends of all the introns and untranslated regions. For *RECQL*, only missense variants in the helicase and RCQ domains (codons 63-592) and exonic truncating variants and are routinely reported. Gross deletion/duplication analysis determines gene copy number for the covered exons and untranslated regions of the sequenced genes and *EPCAM*. For *EPCAM*, only gross deletions encompassing the 3' end of the gene are reported.

Result Reports: Results reported herein may be of constitutional or somatic origin. This methodology cannot differentiate between these possibilities. In result reports, alterations in the following classifications are always reported, and are based on the following definitions and clinical recommendations:

- **Pathogenic Mutation**: alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing of at-risk relatives and appropriate changes in medical management for pathogenic mutation carriers recommended. Previously described pathogenic mutations, including intronic mutations at any position, are always reported when detected.
- **Variant, Likely Pathogenic (VLP)**: alterations with strong evidence in favor of pathogenicity. Targeted testing of at-risk relatives and appropriate changes in medical management for VLP carriers typically recommended. Previously described likely pathogenic variants, including intronic VLPs at any position, are always reported when detected.
- **Variant, Unknown Significance (VUS)**: alterations with limited and/or conflicting evidence regarding pathogenicity. Familial testing via the Family Studies Program recommended. Medical management to be based personal/family clinical histories, not VUS carrier status. Note, intronic VUSs are always reported out to 5 basepairs from the splice junction when detected.

Alterations of unlikely clinical significance (those with strong/very strong evidence to argue against pathogenicity) are not routinely included on results reports. These include findings classified as "likely benign" and "benign" alterations.

Assay Information Continued on Next Page

ASSAY INFORMATION *(Supplement to Test Results - Continued)*

Resources: The following references are used in variant analysis and classification when applicable for observed genetic alterations.

1. The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1092 human genomes. *Nature*. 2012;491:56-65.
2. ACMG Standards and guidelines for the interpretation of sequence variants. *Genet Med.* 2015 May;17(5):405-23.
3. Ambry Genetics Variant Classification Scheme. http://www.ambrygen.com/variant-classification.
4. Berkeley Drosophila Genome Project [Internet]. Reese MG et al. *J Comp Biol.* 1997;4:311-23. http://www.fruitfly.org/seq_tools/splice.html.
5. Database of Single Nucleotide Polymorphisms (dbSNP) [Internet]. Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine (dbSNP Build ID:135) Available from: www.ncbi.nlm.nih.gov/SNP. Accessed Jan 2012).
6. ESEfinder [Internet]. Smith PJ, et al. (2006) *Hum Mol Genet.* 15(16):2490-2508 and Cartegni L, et al. *Nucleic Acid Research.* 2003;31(13):3568-3571. http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home.
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8. Grantham R. Amino acid difference formula to help explain protein evolution. *Science.* 1974;185(4151):862-864.
9. HGMD® [Internet]: Stenson PD et al. *Genome Med.* 2009;1(1):13. www.hgmd.cf.ac.uk.
10. Landrum MJ et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014 Jan 1;42(1):D980-5. doi: 10.1093/nar/gkt1113. PubMed PMID: 24234437.
11. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), Copyright® 1966-2012. World Wide Web URL: http://omim.org.
12. Feng BJ. PERCH: A Unified Framework for Disease Gene Prioritization. *Hum Mutat.* 2017 Mar;38(3):243-251.
13. Exome Aggregation Consortium (ExAC) [Internet], Cambridge, MA. Available from: http://exac.broadinstitute.org.
14. Genome Aggregation Database (gnomAD) [Internet], Cambridge, MA. Available from: http://gnomad.broadinstitute.org.
15. Lek M et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016 Aug 17;536(7616):285-91. PMID: 27535533
16. Mu W et al. *J Mol Diagn*. 2016 Oct 4. PubMed PMID: 27720647
17. Karczewski KJ et al. *Nature*. 2020 May;581(7809):434-443. PMID: 32461654

Disclaimer: This test was developed and its performance characteristics were determined by Ambry Genetics Corporation. It has not been cleared or approved by the US Food and Drug Administration. The FDA does not require this test to go through premarket FDA review. It should not be regarded as investigational or for research. This test should be interpreted in context with other clinical findings. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. This test analyzes the following types of mutations: nucleotide substitutions, small deletions (up to 25 bp), small insertions (up to 10 bp), small indels and gross deletions/duplications. Unless otherwise noted in the methodology section above, it is not intended to analyze the following types of alterations: gross rearrangements, deep intronic variations, Alu element insertions, and other unknown abnormalities. The pattern of mutation types varies with the gene tested and this test detects a high but variable percentage of known and unknown mutants of the classes stated. A negative result from the analysis cannot rule out the possibility that the tested individual carries a rare unexamined mutation or mutation in the undetectable group. This test is designed and validated to be capable of detecting ~99% of described mutations in the 23 genes represented on the panel (analytical sensitivity). The clinical sensitivity of this test may vary widely according to the specific clinical and family history. Breast and gynecologic cancers are complex clinical disorders. Mutations in other genes or the regions not analyzed by this test can also give rise to clinical conditions similar to breast and/or gynecologic cancer. Although molecular tests are highly accurate, rare diagnostic errors may occur. Possible diagnostic errors include sample mix-up, erroneous paternity identification, technical errors, clerical errors, and genotyping errors. Genotyping errors can result from trace contamination of PCR reactions, from maternal cell contamination in fetal samples, from rare genetic variants that interfere with analysis, germline or somatic mosaicism, presence of pseudogenes, technical difficulties in regions with high GC content or homopolymer tracts, active hematologic disease, a history of allogeneic bone marrow or peripheral stem cell transplant, or from other sources. Rare variants present in the human genome reference sequence (GRCh37.p5/hg19) or rare misalignment due to presence of pseudogenes can lead to misinterpretation of patient sequence data**.** This report does not represent medical advice. Any questions, suggestions, or concerns regarding interpretation of results should be forwarded to a genetic counselor, medical geneticist, or physician skilled in interpretation of the relevant medical literature.

Ambry Genetics®

Understanding Your Negative Hereditary Cancer Genetic Test Result

INFORMATION FOR PATIENTS

Result	**NEGATIVE**	Your testing did not find any disease-causing mutations (changes, like spelling mistakes) in the genes tested.
Cancer Risks	**VARIES**	Even though no mutation was found, you may still have an increased risk of developing cancer based on other possible factors, including the following: • Your medical and/or family history • You could have a mutation in the genes tested that cannot be found with current testing methods • You could have a mutation in a gene that has not yet been linked to cancer or was not tested Your healthcare provider can help you learn more about this.
Risk Management	**VARIES**	Risk management decisions are very personal, and depend on many factors. Talk to your healthcare provider about which, if any, options may be right for you.
Family Members	**VARIABLE RISKS**	Depending on your medical and/or family history, your relatives may still have an increased risk of developing cancer and may be eligible for genetic testing and/or increased cancer screening. They should discuss this with a healthcare provider.
Next Steps	**DISCUSS**	Please share this with family members so they can talk with their healthcare providers and learn more. Stay in contact with your healthcare provider for any relevant updates in genetic testing and/or cancer screening. Also, remember to update him/her with any new information about your family history, especially new cancer diagnoses, as this may change how they determine your cancer risks.
Reach Out	**RESOURCES**	• Ambry's Hereditary Cancer Site for Families patients.ambrygen.com/cancer • American Cancer Society cancer.org • Genetic Information Nondiscrimination Act (GINA) ginahelp.org • National Society of Genetic Counselors nsgc.org • Canadian Association of Genetic Counsellors cagc-accg.ca

Please discuss this information with your healthcare provider. The cancer genetics field is continuously evolving, so updates related to your genetic test result, medical recommendations, genetic testing options, and/or potential treatments may be available over time. This information is not meant to replace a discussion with a healthcare provider, and should not be considered or interpreted as medical advice.

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