Breast Cancer High Risk Entended Panel Plus Test Methodology

Medical Diagnostic Laboratories (MDL) is utilizing targeted next-generation sequencing (NGS) technology to test germline variants in the Breast Cancer High Risk Extended Panel Plus genes in its *BRCA*care line of testing.

MDL accepts saliva and blood specimens from ordering healthcare providers for the *BRCA*care line of testing. The *BRCA*care Transport Kit contains all components required for documenting, collecting and transporting a DNA sample: the MDL labeled mailer, a leak-proof zip-lock bag, a Saliva DNA Self-Collection Kit, a Requisition Form, a Patient Consent Form and a Collection Instruction Sheet. DNA quantity is verified upon receipt of the specimen at the laboratory.

Currently, the *BRCA*care testing turn-around-time is 14-21 days. MDL has a policy for immediate management of the emergency cases (i.e., surgical decisions) so that specimen processing and analysis of the results can be completed in a maximum of 10 days.

According to the MDL workflow, DNA is extracted from the specimen in-house before determining any appropriate insurance coverage. If the patient does not proceed with the testing for any reason prior to the initiation of the actual test process, the specimen will be destroyed and the patient will not have any financial obligations.

The MDL *BRCA*care testing has a sensitivity and specificity of > 99.9% after confirmation with Sanger sequencing.

DESCRIPTION OF ANALYSES

Test No.	MDL *BRCA*care Test Options
2600	**Breast Cancer High Risk Extended Panel Plus: 15 genes** - (BRCA1, BRCA2, CDH1, PTEN, TP53, STK11, ATM, CHEK2, PALB2, BARD1, BRIP1, RAD51C, RAD51D, NF1, NBN) by Gene Sequencing with BRCA1/2 Deletion/ Duplication Analysis
2602	**Lynch Syndrome Gene Panel: 5 Genes** - (EPCAM*, MLH1, MSH2, MSH6, PMS2) by Gene Sequencing with Deletion/Duplication Analysis
2601	BRCA1/2: Comprehensive BRCA Analysis by Gene Sequencing with Deletion/ Duplication Analysis
1222	BRCA1/2: Ashkenazi Jewish 3-site Mutation Analysis
1236	BRCA1/2: Ashkenazi Jewish 3-site Mutation Analysis (Reflex to Breast Cancer High Risk Extended Panel Plus) (*If the Ashkenazi Jewish 3-site Mutation Analysis is negative, reflex to 2600)
1224	Gene Specific Site Analysis

MDL Breast Cancer High Risk Extended Panel Plus : The Breast Cancer High Risk Extended Panel Plus test analyzes the entire coding region of the *BRCA1, BRCA2, CDH1, PTEN, TP53, STK11, ATM, CHEK2, PALB2, BARD1, BRIP1, NF1, NBN, RAD51C,* and *RAD51D* genes via comprehensive gene sequencing and gross deletion and duplication (i.e., large rearrangement) analyses. The test also analyzes 10-20 nucleotide bases into the 5' and 3' ends of all intronic and untranslated regions (5'UTR and 3'UTR) for all genes. Clinically-significant intronic findings beyond 5 nucleotide base pairs are also routinely analyzed. Initially, genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using standardized methodologies and quantified using Real-Time PCR. Sequence enrichment is carried out by incorporating the gDNA into microdroplets along with primer pairs designed to target the *BRCA1* and *BRCA2* coding exons and adjacent intronic nucleotides followed by PCR and Ion PGM sequencing. Sanger sequencing, the "gold standard", is performed to confirm the following variants: likely benign, variants of uncertain significance (VUS), likely pathogenic, and pathogenic. Gross deletion and duplication analysis that tests for large rearrangements not traditionally detected by sequence analysis alone is performed using multiple ligand-dependent probe amplification (MLPA).

BRCA1 is located in the long (q) arm of chromosome 17. The MDL comprehensive *BRCA* analysis determines the full sequence in both forward and reverse directions of 5,989 base pairs comprising 22 coding exons and approximately 500 adjacent base pairs beyond the targeted coding exons and the exon-intron boundaries. Exons 1 and 4, which are non-coding exons, are not analyzed. The wild-type *BRCA1* gene encodes a protein comprised of 1,863 amino acids.

BRCA2 is located in the long (q) arm of chromosome 13. The MDL comprehensive *BRCA* analysis determines the full sequence in both forward and reverse directions of 10,257 base pairs comprising 26 coding exons and approximately 1,500 adjacent base pairs beyond the targeted coding exon and exon-intron boundaries. Exon 1, which is a non-coding exon, is not analyzed. The wild-type *BRCA2* gene encodes a protein comprised of 3,418 amino acids.

ATM (ATM serine/threonine kinase) gene is associated with the autosomal recessive condition ataxia-telangiectasia, a disorder characterized by cerebral ataxia, immunodeficiency and increased risk of malignancies, including breast cancer. Heterozygous carriers of ATM mutations have a 2-fold increased breast cancer risk compared to the general population, in women under the age of 50, this risk reaches 5-fold. ATM down-regulation is associated with an aggressive feature and poor prognosis in sporadic breast carcinomas.

BARD1, BRIP1, RAD51C, and RAD51D genes are integral components of the Fanconi anemia-BRCA pathway. Mutations in these genes are estimated to confer up to a 4- and 5-fold increase in the relative risk of breast cancer. RAD51C and RAD51D mutation carriers might benefit from new targeted therapy such as PARP inhibitors due to a deficiency of homologous recombination in RAD51C/ RAD51D carcinomas.

2.2024

CDH1 gene (E-cadherin) germline mutations are associated with Hereditary Diffuse Gastric Cancer (HDGC) with a 67% - 83% lifetime risk for diffuse gastric cancer and from 39% to 52% (for 2398delC mutation) risk for lobular breast cancer. Rates of CHD1 mutations in women with invasive lobular breast cancer without family history of HDGC is low (around 1%) but can be higher (up to 8%) in women with bilateral lobular carcinoma and in women diagnosed younger than age 45. The histology of lobular breast carcinoma is characterized by infiltrative cancer cells which are isolated, highly dispersive and demonstrate a growth pattern with scattered and "single files" of tumor cells dispersed in stromal tissue which is remarkably similar to diffuse gastric cancer. As loss of E-cadherin expression is a distinctive trait of both cancers, it likely contributes to the unique histopathologic features shared by the two cancers demonstrated by characteristic mucinous, signet ring cells.

CHEK2 gene mutations confer an increased risk of developing different types of cancer, including breast, prostate, colon, thyroid, and kidney. Mutation 1100delC has been associated with female and male breast cancer and is particularly frequent in Northern and Eastern European populations (0.7%). A lifetime risk of breast cancer for carriers is as high as 37%, homozygotes have a 6-fold increased risk of breast cancer. The prevalence of 1100delC is higher in female breast cancer cases (1.8% - 1.9%), young-onset breast cancer cases (3.7% in women diagnosed at age <50), and familial breast cancer cases (2.1% - 3.1%). CHEK-2-associated breast cancers tend to be bilateral, estrogen and progesterone-receptors positive, recurrent forms.

NF1 gene is located on chromosome 17q11.2 and encodes the protein neurofibromin. Neurofibromin regulates multiple critical cell-signaling pathways, such as the RAS-cAMP pathway, affecting cell proliferation, survival, growth, migration, and differentiation. Females under the age of 50 with *NF1* gene mutations have an up to five times higher risk of breast cancer morbidity and mortality.

NBN gene is located on chromosome 8q21.3 and encodes the Nibrin protein. Nibrin is responsible for interaction with DNA repair proteins involved in DNA double-strand break signaling. Carriers of *NBN* monoallelic mutations have a significantly increased risk of breast cancer, with an estimated odds ratio of 3.1. Additionally, the truncated c.657del5 variant of *NBN* is also regarded as a high-risk factor for breast cancer.

PALB2 gene (partner and localizer of BRCA2) mutations have been primarily associated with pancreatic and breast cancer, including bilateral female breast cancer and male breast cancer. Relative risk for breast cancer is estimated to be 2.3-fold and higher, and may be mutation dependent.

PTEN gene mutations are associated with PTEN Hamartoma Tumor syndrome (PHTS), which includes Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Proteus and Proteus-like syndrome, Lhermitte-Duclos disease, and autism with macrocephaly. Cowden syndrome is an autosomal dominant condition characterized by mucocutaneous findings and increased risk for both benign and malignant tumors of the thyroid, breast and endometrium. Mutations in the PTEN gene can be found in approximately 85% of individuals with Cowden syndrome. In sporadic breast carcinomas, the frequency of PTEN loss is 30% - 40%. The most well established PTEN-associated cancers include female breast cancer (lifetime risk ranges from 25% to 85%), non-medullary thyroid cancer (3% - 38% risk), endometrial cancer (5% - 28% risk), renal cancer (15% - 34% risk), colorectal cancer (9% - 16% risk), and melanoma (6% risk). PTEN also sensitizes breast cancer to trastuzumab treatment, antagonizing HER2-driven tumorogenesis. PTEN loss and PTEN-independent activation of the PI3K pathway were identified as a major determinant of trastuzumab resistance.

STK11 Serine/threonine kinase 11 (STK11) also known as liver kinase B1 (LKB1) or renal carcinoma antigen NY-REN-19 is a protein kinase that in humans is encoded by the STK11 gene. This gene, which encodes a member of the serine/threonine kinase family, regulates cell polarity and functions as a tumor suppressor. Mutations in this gene have been associated with Peutz-Jeghers syndrome, an autosomal dominant disorder characterized by the growth of polyps in the gastrointestinal tract, pigmented macules on the skin and mouth, and other neoplasms.

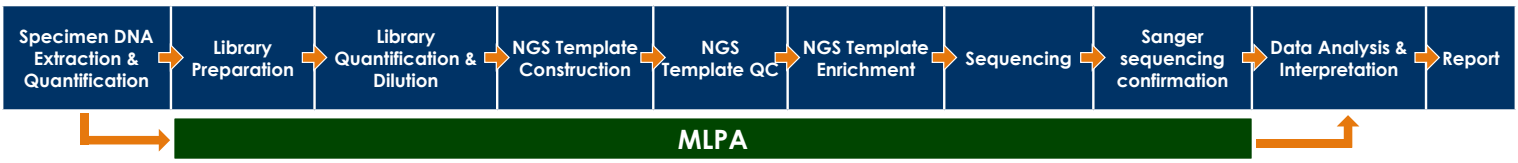
TP53 is a tumor suppressor gene located on chromosome 17p13.1. TP53 mutation remains the most common genetic change identified in human neoplasia. TP53 mutations associated with Li-Fraumeni syndrome have a lifelong risk for the development of different cancers around 90%. The wide spectrum of associated malignancies includes breast cancer, sarcomas, brain cancer, adrenocortical carcinomas, and leukemias with a median age at diagnosis of first malignancy of 25. Patients with Li-Fraumeni syndrome have a breast cancer risk of 56% by the age 45 and greater than 90% by the age of 60, which accounts for a 60-fold increased risk for early onset breast cancer in comparison to the general population. TP53 status may be predictive of outcome in breast cancer and associated with the more aggressive forms and lower survival rate.

MDL Ashkenazi Jewish 3-site Mutation Analysis: The Ashkenazi Jewish population has been found to have two founder-specific mutations in the *BRCA1* gene (185delAG and 5382insC) and one founder-specific mutation in the *BRCA2* gene (6174delT). Cancer epidemiological studies have reported that 78% to 96% of Ashkenazi Jews with detectable mutations carry one of the founder mutations. Sanger DNA sequencing is performed to analyze these specific genetic hot-spot locations.

MDL *BRCA1* and *BRCA2* Specific Site Analysis: DNA sequencing is performed for a specified *BRCA1* and/or *BRCA2* mutation (known familial *BRCA1* and/or *BRCA2* variant). The specific mutation to be analyzed must be provided with the physician-completed test requisition.

THE MDL BREAST CANCER HIGH RISK EXTENDED PANEL PLUS WORKFLOW

MDL has developed an Ion Torrent PGM-based routine diagnostic procedure for *BRCA1* and *BRCA2* germline variant detection.



TEST LIMITATIONS

The MDL genetic test determines the presence of mutations in only select important gene(s) associated with Hereditary Breast and Ovarian Cancer (HBOC) syndrome. This assay cannot detect mutations affecting genes regions not examined in the assay. Intronic regions are analyzed up to 20 nucleotides before and 10 nucleotides after each intron/exon boundary.

This test is not the only way to detect genetic abnormalities. The patient's healthcare provider may also recommend other genetic, imaging and/or laboratory tests based on the personal/family cancer history and clinical information.

BREAST CANCER HIGH RISK EXTENDED PANEL PLUS W TEST RESULTS INTERPRETATION

As a College of American Pathologists (CAP)-accredited laboratory, MDL utilizes the standard HGVS nomenclature for Breast Cancer High Risk Extended Panel Plus test reports, for example:

Test Performed	Reference Sequence Used	Common mutation name	Mutation Nucleotide Change	Mutation Amino Acid Change	Exon number	Interpretation / Comments
BRCA1 Sequencing	NC.000017.10	185delAG	c.68_69delAG	p.Glu23Valfs*17	2	**PATHOGENIC**

Results are interpreted and reported following the recommendations of the American College of Medical Genetics (ACMG) as a guideline (www.acmg.com). The overall possible result categories of the hereditary cancer genetic testing are negative, positive or uncertain.

Report label	Test Result Category
	Positive Test Result: **(Pathogenic or Likely Pathogenic Variant)** A mutation in a gene(s) associated with an increased risk for hereditary cancer was identified.
	Uncertain Test Result: **(Variant of Uncertain Significance, VUS)** Genetic alteration(s)/change(s) were detected, but it is not known if these changes pose a cancer risk based on the current scientific information.
	Negative Test Result: **(Benign or Likely Benign Variant)** No harmful mutation was identified.

New variants are classified based upon the current literature and publicly integrated databases. The information from these databases is incorporated into each patient report and presented to the healthcare provider for final risk assessment. MDL utilizes the following well-known publicly integrated informational resources to determine variant classification:

Population databases:

- 1000Genomes (http://brower.1000genomes.org)
- NHLBI GO Exome Sequencing Project (ESP) (http://evs.gs.washington.edu/EVS)
- dbSNP (http://www.ncbi.nlm.nih.gov/snp)

Disease database:

- ClinVar (http://www.ncbi.nlm.nih.gov/clinvar)
- BIC (http://www.genome.gov/page.cfm?pageID=10000608)

In Silico analysis tools:

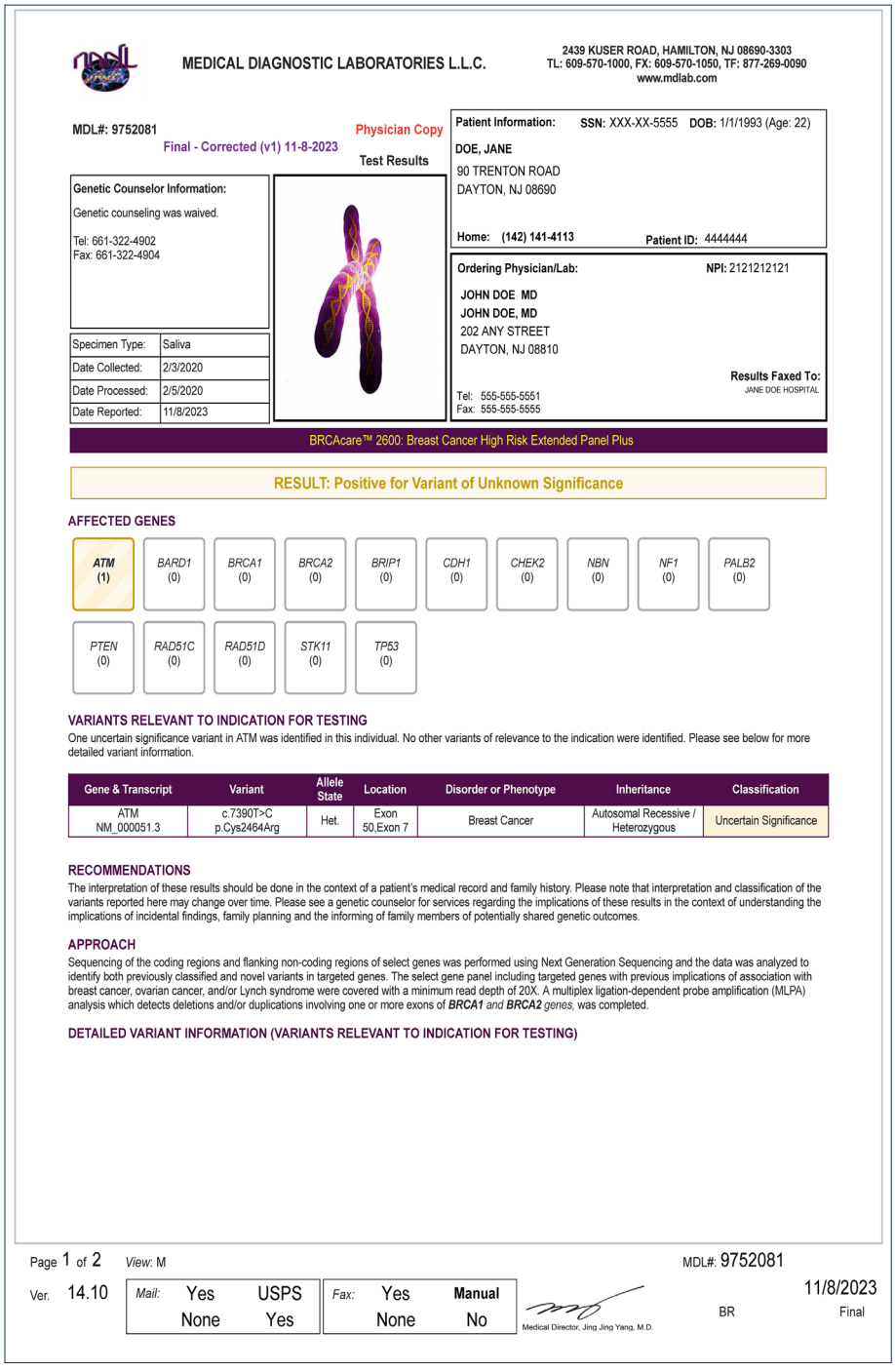
- Protein Variation Effect Analyzer (PROVEAN) (http://provean.jcvi.org/index.php)
- SIFT (http://sift.jcvi.org/)
- Mutation Taster (http://www.mutationtaster.org/)

For Breast Cancer High Risk Extended Panel Plus variant classification, MDL uses an internal interpretation algorithm "Variant Classification System for BRCA Genetic Testing". The MDL variant classification system, based on the 5-tier system recommendations for the interpretation of sequence variants proposed by the American College of Medical Genetics and Genomics (ACMG), complies with the standards and guidelines for the interpretation of sequence variants by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) (Genet Med. 2015 May; 17(5):405-24). MDL also utilizes in silico methods (PROVEAN, SIFT and Mutation Taster) to partially characterize previously unreported variants. This process allows MDL to provide information on the predicted effect of missense changes on protein structure and function. Internally, each variant is extensively analyzed and recorded per the MDL Variant Classification System. Once a variant classification is internally selected, this classification will be used for future observations of the variant until a modification is reflected in the databases.

The classification and interpretation of all genetic variants identified as a result of this genetic testing is based on the currently available scientific information, plus supplementation with internal analysis as described above for unreported variants. As new scientific information becomes available, in some circumstances, the classification and interpretation of the genetic variants may change. Accordingly, if reported variants are reclassified, an updated patient result will be issued to the healthcare provider on a quarterly basis.

In coordination with the healthcare provider and genetic counselors, MDL provides complimentary testing of gene variants of uncertain significance (VUS) for blood relatives with hereditary breast and ovarian cancer syndrome (HBOC) according to the National Comprehensive Cancer Network HBOC Guidelines (version 2.2014). This is offered to patients for which primary detection of their VUS was identified by the MDL Breast Cancer High Risk Extended Panel Plus. This information will aid in variant classification and will be made publically available through deposition into the ClinVar and BIC databases.

Breast Cancer High Risk Entended Panel Plus



MEDICAL DIAGNOSTIC LABORATORIES L.L.C.

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MDL#: 9752081
Final - Corrected (v1) 11-8-2023

Physician Copy
Test Results

Patient Information: SSN: XXX-XX-5555 DOB: 1/1/1993 (Age: 22)
DOE, JANE
90 TRENTON ROAD
DAYTON, NJ 08690
Home: (142) 141-4113 Patient ID: 4444444

Genetic Counselor Information:
Genetic counseling was waived.
Tel: 661-322-4902
Fax: 661-322-4904

Specimen Type:	Saliva
Date Collected:	2/3/2020
Date Processed:	2/5/2020
Date Reported:	11/8/2023

Ordering Physician/Lab: NPI: 2121212121
JOHN DOE MD
JOHN DOE, MD
202 ANY STREET
DAYTON, NJ 08810
Tel: 555-555-5551
Fax: 555-555-5555

Results Faxed To:
JANE DOE HOSPITAL

BRCAcare™ 2600: Breast Cancer High Risk Extended Panel Plus

RESULT: Positive for Variant of Unknown Significance

AFFECTED GENES

***ATM* (1)**	*BARD1* (0)	*BRCA1* (0)	*BRCA2* (0)	*BRIP1* (0)	*CDH1* (0)	*CHEK2* (0)	*NBN* (0)	*NF1* (0)	*PALB2* (0)
PTEN (0)	*RAD51C* (0)	*RAD51D* (0)	*STK11* (0)	*TP53* (0)					

VARIANTS RELEVANT TO INDICATION FOR TESTING

One uncertain significance variant in ATM was identified in this individual. No other variants of relevance to the indication were identified. Please see below for more detailed variant information.

Gene & Transcript	Variant	Allele State	Location	Disorder or Phenotype	Inheritance	Classification
ATM NM_000051.3	c.7390T>C p.Cys2464Arg	Het.	Exon 50,Exon 7	Breast Cancer	Autosomal Recessive / Heterozygous	Uncertain Significance

RECOMMENDATIONS

The interpretation of these results should be done in the context of a patient's medical record and family history. Please note that interpretation and classification of the variants reported here may change over time. Please see a genetic counselor for services regarding the implications of these results in the context of understanding the implications of incidental findings, family planning and the informing of family members of potentially shared genetic outcomes.

APPROACH

Sequencing of the coding regions and flanking non-coding regions of select genes was performed using Next Generation Sequencing and the data was analyzed to identify both previously classified and novel variants in targeted genes. The select gene panel including targeted genes with previous implications of association with breast cancer, ovarian cancer, and/or Lynch syndrome were covered with a minimum read depth of 20X. A multiplex ligation-dependent probe amplification (MLPA) analysis which detects deletions and/or duplications involving one or more exons of ***BRCA1*** *and* ***BRCA2*** *genes,* was completed.

DETAILED VARIANT INFORMATION (VARIANTS RELEVANT TO INDICATION FOR TESTING)

Page 1 of 2 *View:* M MDL#: 9752081

Ver. 14.10

Mail:	Yes	USPS	*Fax:*	Yes	**Manual**
	None	Yes		None	No

Medical Director, Jing Jing Yang, M.D.

BR 11/8/2023 Final