Patient Name	Centre
Age/Gender	OP/IP No/UHID
MaxID/Lab ID	Collection Date/Time
Ref By	Reporting Date/Time

TEST REQUESTED: BRCA1 & BRCA2 MUTATION DETECTION WITH MLPA

METHOD USED

Next Generation Sequencing & MLPA

SAMPLE INFORMATION

Sample Type: Whole Blood

NEGATIVE RESULT

No Pathogenic Mutation Detected/No CNV Detected

TEST RESULT SUMMARY

Next Generation Sequencing	MLPA (Copy Number Variation, CNV)
NEGATIVE	NEGATIVE

INTERPRETATION

- Next Generation sequencing did not identify any clinically significant variants in the coding regions or splice site of *BRCA1* and *BRCA2* genes in this sample.
- Multiplex Ligation Probe Assay did not identify any CNV in *BRCA1* & *BRCA2*.
- A negative test result reduces but does not eliminate the possibility that this carcinoma has a genetic cause, as it may be due to a variant in a genomic region not covered by the test.
- No other variant that warrants to be pathogenic was detected. Variations with high minor allele frequencies which are benign/likely benign will be given upon request.

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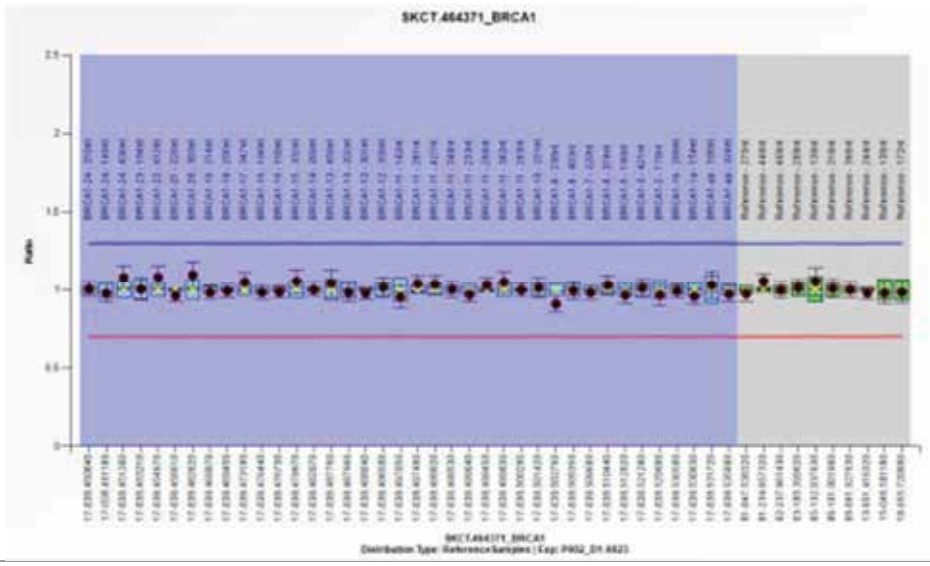
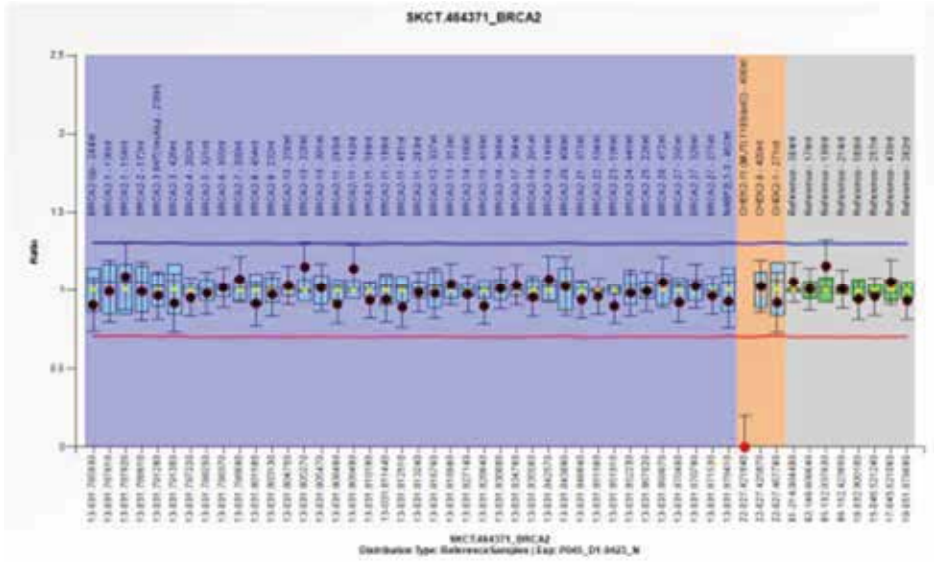
MULTIPLEX LIGATION PROBE ASSAY

CNV was not detected in *BRCA1* gene by MLPA.



CNV was not detected in *BRCA2* gene by MLPA.



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SAMPLE STATISTICS (Targeted Nucleotides Covered)	
Coverage	100%
Depth	4,492x

Adopted rules to classify sequence variants based on American College of Medical genetics recommendations (2015):

INTERPRETATION		
ACMG Criteria/Tier classification	**Reporting Criteria**	**Remarks**
Benign/ Tier IV	No Clinically Significant Mutation Detected	Variants that are not causative for a disease and are present at higher frequency in general population
Likely Benign/ Tier IV	No Clinically Significant Mutation Detected	Variants with possible neutral effects and diagnosis not confirmed
Uncertain Significance/ Tier III	Uncertain Significance	Significance to the function or health of an organism is not known. Significance can be assessed/altered with time, subject to availability of scientific evidence
Likely Pathogenic/ Tier II	Likely Pathogenic	Variants where the evidence is compelling, but not definitive, to cause disease
Pathogenic/ Tier I	Pathogenic	Variants that are well-documented to cause disease

Cancer Risk for BRCA1/BRCA2 clinically significant variant:

CANCER TYPE	CANCER RISK (BRCA1/BRCA2)	RESOURCE
Ovarian Cancer (70Y-80Y)	39%-44% /11%-17%	https://www.cancer.gov/
Breast Cancer (Female) (70Y-80Y)	55%-72% /45%-69%	https://www.cancer.gov/
Breast Cancer (Male) (70Y-80Y)	1%/7%	https://www.cancer.net/
Pancreatic Cancer	1%/2%	https://ascopubs.org/
Prostate Cancer	<1.2%-3.2% /1.2%-3.2%	https://ascopubs.org/

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TEST METHODOLOGY

DNA was extracted and BRCA library was prepared using Devyser BRCA Library preparation kit. This panel is composed of coding regions and adjacent exon-intron boundaries in the *BRCA1* and *BRCA2* genes. Specifically, for BRCA1 the Devyser BRCA kit offers full sequence determination of all 23 coding exons of transcript NM_007300 and all 22 coding exons of LRG_292t1. Similarly, for BRCA2 the Devyser BRCA kit offers full sequence determination of all 26 coding exons of transcript NM_000059 and LRG_293t1. Additionally, all adjacent exon-intron boundaries including minimum 14 bp proximal to the 5' end and 10bp distal to the 3' end of each exon boundary are covered. Target specific primers are designed such that primer footprints are covered by an overlapping amplicon to enable detection of primer site SNVs. The Devyser BRCA libraries were sequenced in paired end mode (2x151cycles). The number of quality filtered sequencing reads, mapped and aligned against the target region, were counted and compared to the total number of reads. Before proceeding to data interpretation, minimal coverage per amplicon was confirmed. For detection of sequence variants of germline origin, it was ensured that each amplicon has at least 100x coverage. Clinically relevant mutations were classified using published variants in literature and a set of diseases databases such as SNPEDIA, UMD BRCA1/BRCA2 database and ClinVar database. Common variants are filtered on allele frequency in 1000Genome phase 3, ExAc (v1.0), gnomAD, dbSNP. In-silico prediction is done to determine the effect of variants using tool such as Polyphen-2, SIFT, etc. Only non-synonymous and splice site variants relevant to the panel focused genes were used for clinical interpretation. Silent variations/ synonymous variant that do not result in any change in amino acid in the coding region are not reported.

This panel also enable us to detect exonic deletion or duplication (CNVs) along with the SNVs by MLPA. Multiplex Ligation dependent Probe Amplification (MLPA) is a multiplex PCR method used to detect abnormal copy numbers in specific and previously known regions of the genome that are associated with a specific genetic condition.

DISCLAIMER

A Negative NGS result implying non-detection of mutation/deletion indicates a Benign/likely Benign polymorphism. These results reduce but do not eliminate the possibility of hereditary cancer as rare genetic abnormalities may not be detected by this assay. Thus a negative result is not very informative when the carrier status of other family members is either unknown or negative. A negative test result may also be due to the inherent technical limitations of the assay. A negative MLPA result indicates that no disease-causing deletions or duplications were identified in the test performed. It does not guarantee that the individual will be healthy or free from other genetic disorders or medical conditions. The combination of a deletion on one chromosome and a similar sized duplication on the other chromosome may result in false negative CNV results.

Results obtained using Devyser BRCA should be interpreted with consideration of the overall picture obtained from clinical and laboratory findings. Sequence variants present in genes other than the *BRCA1* and *BRCA2* genes will not be detected using Devyser BRCA. Only variations in genes potentially related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or positive results. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

A Deleterious / Suspected deleterious result indicates a likely pathogenic mutation with an increased risk for BRCA1 or BRCA2 associated cancer including breast, ovarian, fallopian tube or peritoneal in a female. In a male it indicates an increased risk of breast or prostate cancer. Genetic testing of close family members is recommended.

The accuracy and completeness of this information may vary due to variable information available in different databases. Classification of the variant may change overtime. An updated variant classification may be obtained on request. Synonymous mutations were not considered while preparing the report. The mutations have not been confirmed using Sanger sequencing and/or alternate technologies and additional testing might be required if clinically indicated.

The information provided should only be utilized as a guide or aid and the decision to select any therapy option based on the information reported here resides solely with the discretion of the treating physician. Patient care and treatment decisions should only be made by the physician after taking into account all relevant information available including but not limited to the patient's condition, family history, findings upon examination, results of other diagnostic tests, and the current standards of care.

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