ClinLab
Clinical Testing Laboratory, Inc.

Clinical Testing Lab of Washington
2150 Pennsylvania Avenue NW
Washington, DC 20037
Phone: 202-555-1212

Specimen Number	Specimen Type	Control Number	Account Number	Account Phone Number	Route
	Peripheral Blood				

Patient Last Name	Patient Barcode

Patient First Name	Patient Middle Name
Leslie	

Patient SS#	Patient Phone	Total Volume

Age (Y/M/D)	Date of Birth	Sex	Fasting
40 y.o.		Female	

Patient Address	Indication / Family History / Ethnicity
	Indication: Bleeding and abdominal pain – suspected Lynch Syndrome **Family History:** Maternal family line – high incidence of varied cancers **Ethnicity:** Mixed race – Japanese & European Caucasian

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Physician ID
			Jane Ferreiro, MD		

Tests Ordered
Lynch Syndrome Mutation Evaluation

General Comments
Please send a copy of the final report to the Molecular Science/M1 Training office via Fax at (202) 555-1212

Clinical test results for Lynch Syndrome Mutation Evaluation

GENE	TEST RESULTS	EXPLANATION
MSH2 (2p21-16.3)	p.Glu48 p.Glu48Ter	**This result confirms the high risk for Lynch Syndrome, as this is a known pathogenic, autosomal dominant variant.** This result should be interpreted in the context of clinical presentation and results of other laboratory tests and biopsy. A PCR/sequencing study has detected one copy of the p.Glu48Ter (MSH2: g.5210G>T, c.-30-27G>T or p.Glu48Ter) variation. The p.Glu48Ter variation causes a premature stop (termination) codon at amino acid position 48 resulting in an abnormally short or truncated protein.
MSH2 (2p21-16.3)	g.4951T g.4951T>C	**This result indicates the presence of a variant that is currently identified as benign for Lynch Syndrome.** Please note that knowledge about variants is constantly evolving and the presence of a benign variant does not rule out the possibility of Lynch Syndrome due to the presence of other potential variants. This result should be interpreted in the context of clinical presentation and results of other laboratory tests and biopsy.
Other genes tested: negative for the presence of known variants.		**EPCAM (2p21)** **MLH1 (3p22.2)** **MSH6 (2p16.3)** **PMS2 (7p22.1)**

INDICATIONS FOR TESTING

Lynch syndrome is characterized by an increased risk for colorectal cancer (CRC) and cancers of the endometrium, ovary, stomach, small bowel, urinary tract, biliary tract, brain (usually glioblastoma), skin (sebaceous adenomas, sebaceous carcinomas, and keratoacanthomas), pancreas, and prostate. Cancer risks and age of onset vary depending on the associated gene. Several other cancer types have been reported to occur in individuals with Lynch syndrome (e.g., breast, sarcomas, adrenocortical carcinoma).

METHODOLOGY

The Cancer Screening Panel uses state-of-the-art sequencing technology to provide high quality results. Genomic DNA is extracted from dry buccal swabs using magnetic particle processing. DNA from patient samples are amplified with

primers specific for the targeted regions using Oligo Directed Patch PCR (Varley, et. al.). Positive and negative controls are used with each run. Barcoded patient samples and positive controls are paired-end sequenced using Illumina NextGen sequencing technology. Targeted sequencing is performed on the entire coding region and intronic/exonic boundaries. Sequences are aligned to the human reference genome and variants (Small Nucleotide Variations, Insertions and Deletions) are called. Large indels (>50 bp), rearrangements, rare abnormalities and structural variations may not be detected. Rare diagnostic errors may occur if variations occur in primer site locations. The regions sequenced include many, but not all, genes that have been shown to affect our risk of developing cancer and/or impact medical management. The following genes are sequenced: EPCAM, MLH1, MSH2, MSH6 and PMS2. The variants analyzed rule out the majority of the abnormalities known to be associated with inherited cancer predisposition in these genes.

PERFORMANCE

All NGS Panels provided by are sliced from custom high-quality whole exome sequencing. Panels cover all coding exons, exon-intron boundaries (± 20 bps) and selected non-coding, deep intronic variants of the genes included in the panel unless otherwise stated in the panel description provided at our website. Our laboratory-developed test has been independently validated. Mean sequencing depth at exome level was 174x and 99.4% of the targeted nucleotides had >20x sequencing coverage. The sensitivity for SNVs was 99.65% (412,456/413,893) and for indels with varying length: 1-10 bps 96.9% (17070/17608), 11-20 bps 98.9% (791/800), 21-30 bps 100.0% (145/145), 31-50 bps 100.0% (19/19). By sequence analysis, the longest detected insertion and deletion were 221 and 210 base pairs, respectively. Sequencing coverage of the target regions was assessed using 31 reference samples from Coriell that were also used for SNV and indel validation. Validation of copy number variant (CNV) detection was performed using clinical samples (small CNVs, n=52) and cell line samples from Coriell (n=37). Sensitivity of small CNVs were 92.3% (24/26) for 1 exon level deletion, 100.0% (11/11) for 2 exons level CNV and 93.3% (14/15) for 3-7 exons CNV. Sensitivity of large CNVs was 100% (n=37, size range (0.01-47 Mb). In the analytic validation, specificity was >99.9% for most variant types. Additional clinical validation showed sensitivity of 100.0% for both sequence variants (n=42) and CNVs (n=63) using different sample types (dried blood spot, blood, saliva, DNA). All clinical samples represented pathogenic and likely pathogenic variants confirmed also by other laboratory assays.

LIMITATIONS

The sequence analysis will not detect mutations located in regions that are not analyzed. The sequencing method also will not detect gross genetic alterations including most duplications, inversions, or deletions (in females). Some sequence alterations that may be detected (such as those causing missense or synonymous changes) will be of unknown clinical significance.

CLINICAL DESCRIPTION

Lynch syndrome, often called hereditary nonpolyposis colorectal cancer (HNPCC), is an inherited disorder that increases the risk of many types of cancer, particularly cancers of the colon (large intestine) and rectum, which are collectively referred to as colorectal cancer. People with Lynch syndrome also have an increased risk of cancers of the stomach, small intestine, liver, gallbladder ducts, urinary tract, brain, and skin. Additionally, women with this disorder have a high risk of cancer of the ovaries and lining of the uterus (endometrial cancer). Women with Lynch syndrome have a higher overall risk of developing cancer than men with the condition because of these cancers of the female reproductive system. In individuals with Lynch syndrome who develop cancer, the cancer typically occurs in their forties or fifties.

People with Lynch syndrome may occasionally have noncancerous (benign) growths in the colon, called colon polyps. In individuals with this disorder, colon polyps occur at a younger age but not in greater numbers than they do in the general population. [from MedlinePlus Genetics]