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PATIENT REPORT

Patient Age/Sex: Female

Specimen Collected: 13-Mar-24 13:06

Mismatch Repair IHC with Reflex to BRAF | **Received: 13-Mar-24 13:06** | **Report/Verified: 13-Mar-24 13:07**

Procedure	Result	Units	Reference Interval
Mismatch Repair by IHC,Result	Abnormal t1 i1		
Mismatch Repair by IHC with MLH1	Abnormal		
Mismatch Repair by IHC with MSH2	Normal		
Mismatch Repair by IHC with MSH6	Normal		
Mismatch Repair by IHC with PMS2	Normal		
Client Case or Ref #	C-55		
MSI Tissue Source	Colon		

BRAF Reflex to MLH1 Promoter Meth | **Received: 13-Mar-24 13:06** | **Report/Verified: 13-Mar-24 13:08**

Procedure	Result	Units	Reference Interval
BRAF REFL Int	Not Detected i2		
Block ID	C-55		

Interpretive Text

t1: 13-Mar-24 13:06 (Mismatch Repair by IHC, Result)

Abnormal immunohistochemical staining for mismatch repair proteins correlates well with the presence of microsatellite instability by PCR. Controls worked appropriately.

This result has been reviewed and approved by [redacted]

Test Information

i1: Mismatch Repair by IHC, Result

INTERPRETIVE INFORMATION: Mismatch Repair by IHC, Result

Immunohistochemical staining for mismatch repair proteins can be used as a surrogate test for microsatellite instability as measured by PCR. Normal results correlate well with the absence of microsatellite instability, while abnormal results correlate well with the presence of microsatellite instability. Abnormal results may also qualify patients for immune checkpoint inhibitor treatment. The immunohistochemical staining pattern can also be used as a guide for the subsequent germline evaluation of mismatch repair genes (refer to Lynch Syndrome - HNPCC) testing algorithm at ARUPconsult.com). Normal staining results consist of any level of staining in the tumor cells (unless evidence of clonal loss). Abnormal staining results consist of complete loss of staining in the tumor cells, in the presence of retained staining in normal (non-tumor) cells, which serve as an internal control. An abnormal overall result may qualify patients for immune checkpoint inhibitor treatment, in the appropriate clinical setting.

Genetic counseling is recommended for the interpretation of all results.

Test Information

i1: Mismatch Repair by IHC, Result

Assay is performed on formalin fixed paraffin-embedded tissue. Antibody clone for MLH1 is ES05, MSH2 is FE11, MSH6 is EP49, and PMS2 is EP51. Detection system is a proprietary polymeric HRP.

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the US Food and Drug Administration. This test was performed in a CLIA certified laboratory and is intended for clinical purposes.

i2: BRAF REFL Int

BACKGROUND INFORMATION: BRAF Mutation Detection with Reflex to MLH1 Promoter Methylation

CHARACTERISTICS: This assay is an amplicon enrichment-based massively parallel sequencing assay targeting hotspot variants in genes critical for the diagnostic, prognostic, and therapeutic assessment of various solid tumors. The amplicon primer pool is designed to interrogate different DNA variant classes including single nucleotide variants (SNVs), multiple nucleotide variants (MNVs), and small insertions and deletions (1-25 base pairs [bp]) within a limited set of highly clinically relevant gene loci for the identification of actionable somatic variants in FFPE tissue from solid tumors.

The presence of a BRAF c.1799T>A, p.Val600Glu (V600E) mutation in a microsatellite unstable colorectal carcinoma indicates that the tumor is likely sporadic and not associated with Lynch syndrome. However, if a BRAF V600E mutation is not detected, the tumor may either be sporadic or Lynch syndrome-associated. It should be noted that there have been rare reports of BRAF mutations in Lynch syndrome-associated tumors, and as such the presence of a BRAF mutation does not completely exclude the possibility of Lynch syndrome.

GENES TESTED: BRAF (NM_004333) exon 15 (chr7:140453100-140453172) is evaluated to detect hotspot SNVs, MNVs, small insertions, and small deletions. This exon is partially covered for hotspots only and not reported in full.

METHODOLOGY: Genomic DNA was isolated from a microscopically-guided dissection of FFPE tumor tissue and then enriched for the targeted regions of the tested genes. The variant status of the targeted genes was determined by massively parallel sequencing. The hg19 (GRCh37) reference sequence was used as a reference for identifying genetic variants. Clinically significant variants and variants of uncertain significance within the preferred transcript are reported.

LIMITATIONS: This test will not detect variants in areas outside the targeted genomic regions or below the limit of detection. Copy number alterations (losses or amplifications), translocations, microsatellite instability, tumor mutational burden, deep intronic variants, and insertions/deletions larger than 25bp will not

Test Information

i2: BRAF REFL Int

be detected. Since this is a DNA-based assay, RNA variants will not be detected. This test evaluates for variants in tumor tissue only and cannot distinguish between somatic and germline variants. Therefore, if a hereditary/familial cancer is of clinical concern, additional clinical evaluation and genetic counseling should be considered prior to additional testing. In some cases, variants may not be identified due to technical limitations related to the presence of known pseudogenes, GC-rich regions, repetitive or homologous regions, low mappability regions, and/or variants located in regions overlapping amplicon primers. Tissue samples yielding between 1ng and 5ng total DNA input may yield suboptimal results and will be accepted for testing with a client-approved disclaimer. Benign or likely benign variants in the preferred transcript are not reported. Variant allele frequency (VAF) is not reported. Additional evaluation should be considered for complete genetic analysis, including detection of variants outside of the hotspot region of BRAF, variants within other genes, translocations, or gene rearrangements, if clinically indicated.

LIMIT OF DETECTION (LOD): The LOD for this assay is 10 percent VAF for all variant classes detected by the assay. For variants near the assay LOD, positive percent agreement (PPA) was found to be greater than 90 percent for all variant classes.

ANALYTICAL ACCURACY/SENSITIVITY (PPA): The PPA estimates for the respective variant classes (with 95 percent credibility region) are listed below. Genes included on this test are a subset of a larger methods-based validation from which the PPA values are derived.
Single nucleotide variants (SNVs): 98.4 percent (95.1-99.7 percent)
Deletions (1-25bp): 96.6 percent (89.6-99.3 percent)
Insertions/duplications (1-25bp): 96.8 percent (90.2-99.3 percent)
Multiple nucleotide variants (MNVs): 98.2 percent (91.8-99.8 percent)

CLINICAL DISCLAIMER: Results of this test must always be interpreted within the context of clinical findings and other relevant data and should not be used alone for a diagnosis of malignancy, determination of prognosis, or recommendation of therapy. This test is not intended to detect minimal residual disease.

This test was developed and its performance characteristics determined by ARUP Laboratories. It has not been cleared or approved by the U.S. Food and Drug Administration. This test was performed in a CLIA-certified laboratory and is intended for clinical purposes.