BRAF V600 mutation detection by Droplet Digital PCR

The UNC Medical Center *Molecular Genetics Laboratory* performs *BRAF* gene testing by Droplet Digital PCR to detect any of three activating mutations in codon 600 (encoding V600E, V600K, or V600R). Results assist in diagnosis, classification and/or treatment selection for solid tumors or hairy cell leukemia.

Rationale for testing:

An activating *BRAF* somatic gene mutation stimulates the BRAF-MEK-ERK biochemical pathway, contributing to cell proliferation and survival. The most relevant mutation, accounting for ~90% of activating mutations, encodes BRAF V600E protein having >10-fold more kinase activity than its normal counterpart.

Detection of an activating *BRAF* mutation in tumor tissue may assist in selecting treatment for a wide range of neoplasms including colorectal adenocarcinoma, melanoma, anaplastic thyroid carcinoma and other advanced solid tumors in adults or children ≥6 years old. When urgent treatment decisions are warranted for a patient with locally advanced or metastatic solid tumor, this droplet digital PCR (ddPCR) assay aims for rapid assessment of actionable *BRAF* mutations.

Additionally, this test can assist in diagnosis of hairy cell leukemia or classification of thyroid neoplasia. Finally, presence of somatic *BRAF* mutation in colon adenocarcinoma tissue diminishes the likelihood of Lynch syndrome (a heritable cancer predisposition syndrome).

Clinical Indications for this *BRAF* V600 mutation test:

1. Predict response to targeted therapy in patients with an advanced solid tumor
2. Aid in diagnosis or classification of certain forms of neoplasia (e.g., hairy cell leukemia, papillary thyroid carcinoma)
3. Risk of Lynch syndrome in patients with colorectal or endometrial carcinoma with MSI-H or dMMR, although *MLH1* promoter hypermethylation remains the preferred test in this situation

Specimen Requirements: The preferred specimen is ten unstained paraffin sections (5-10µM thick, plain glass), plus an H&E-stained slide on which areas with >10% malignant cells are circled with a total area >2mm2. Unfixed marrow aspirate smears or biopsy touch preparation slides are also acceptable. Specimens having 5-10% malignant cells are considered at the discretion of a lab director. Unacceptable specimen types are plasma and frozen or decalcified tissue. A copy of the pathology report is requested.

Lab Method: Droplet digital polymerase chain reaction (ddPCR, Bio-Rad system) detects three *BRAF* gene mutations: c.1799T>A (p.V600E), c.1798_1799delinsAA (p.V600K), and c.1798_1799delinsAG (p.V600R). Other rare DNA variants that may activate *BRAF* are not detectable. Results are interpreted by a pathologist and reported as positive or negative to a sensitivity of 1% allele fraction (equivalent to 2% cells with a heterozygous mutation). Variants are reported relative to NM_4333.6 and NP_004324.2 reference sequences. The reference value is 'No BRAF V600 mutation detected'.

References:

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Questions? Call the UNC *Molecular Genetics Lab* at (984) 974-1825 or visit the website at: Molecular Genetics | McLendon Clinical Laboratories | UNC Medical Center