



Molecular Diagnostics Laboratory Test Directory

Test Name	Assay Description	Assay Type	Specimen Type Accepted	Category/Use
B Cell Clonality	IGH and IGK locus gene rearrangements (PCR/CE)	Qual	Blood BMA CSF FNA FFPE Tissue	Hematopathology/ Diagnosis
T Cell Clonality	T cell receptor gamma locus (TRG) gene rearrangements (PCR/CE)	Qual		
IGH@/BCL2 (MBR)	Translocation detection at major breakpoint region (real-time qPCR)	Qual		
EBV (EBNA2)	Lymphoma-associated virus detection (real-time qPCR)	Qual		
HHV8	PEL, Castleman's disease, Kaposi's Sarcoma-associated virus detection (real-time qPCR)	Qual		
HTLV-I (Pol)	Human T-cell leukemia virus detection (real-time qPCR)	Qual		
MYD88	Detection of MYD88 c.794T>C, p.L265P (Clamp-PCR/pyrosequencing)	Qual	Blood BMA FFPE Tissue	
Desmoplastic Round Cell Sarcoma	EWSR1/WT1, t(11;22) translocation detection (real-time qRT-PCR)	Qual	FFPE Tissue	Sarcoma/ Diagnosis
Ewing's Sarcoma	EWSR1/ERG, t(21;22) EWSR1/FLI1 Type1, t(11;22) EWSR1/FLI1 Type2, t(11;22) translocation detection (real-time qRT-PCR)	Qual		
Rhabdomyosarcoma Sarcoma	PAX3/FOXO1, t(2;13) PAX7/FOXO1, t(1;13) translocation detection (real-time qRT-PCR)	Qual		
Synovial Sarcoma	SYT/SSX1, t(X;18) SYT/SSX2, t(X;18) translocation detection (real-time qRT-PCR)	Qual		

Cancer Gene Mutation-Standard Panel (a multiplex 50 gene panel with next-generation sequencing)	Detection of 2850 COSMIC hotspot mutations in 50 cancer genes (ABL1, AKT1 , ALK, APC, ATM, BRAF , CDH1, CDKN2A, CSF1R, CTNNB1, EGFR , ERBB2 , ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3 , FLT3, GNA11 , GNAQ , GNAS, HNF1A, HRAS , IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS , MET, MLH1, MPL, NOTCH1, NPM1, NRAS , PDGFRA, PIK3CA , PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53 , VHL) using Ion AmpliSeq Cancer Hotspot Panel v2 on Ion Torrent PGM	Qual	FFPE Tissue	NSCLC, Colorectal Cancer, Bladder Cancer, Melanoma /Oncology Clinical Research Protocol
BRAF V600E Mutation	Detection of BRAF c.1799T>A, V600E mutation with an ultra-sensitive castPCR assay. The limit of detection is about 0.05% mutant allele.	Qual/ Quant	Blood BMA CSF FNA FFPE Tissue	Erdheim-Chester disease, Papillary thyroid carcinoma, Hairy cell leukemia /Diagnosis or Oncology Clinical Research Protocol
EGFR Germline Mutation	Detection of EGFR germline mutations T790M, R776G, R831C, V841I (pyrosequencing)	Qual	Blood	NSCLC Genetic Study, protocol # 11-C-0096
HABP2 Germline Variant	Detection of HABP2 germline variant G534E (pyrosequencing)	Qual	Blood	Familial non-medullary thyroid cancer (FNMTC) trial, protocol # 10-C-0102
MGMT Methylation	Methylation analysis of 12 CpG sites in exon 1 (pyrosequencing)	Qual	FFPE Tissue	Glioblastoma/ Neuro-oncology Clinical Research Protocol
IDH1/IDH2	Detection of mutations in IDH1 codon 132 and IDH2 codon 172 (pyrosequencing or NGS)	Qual		
EGFRvIII RT-PCR	Detection of EGFR variant III expression (real-time qRT-PCR)	Qual	FFPE Tissue	Glioblastoma/ Surgery Branch Protocol of Anti-EGFRvIII CAR PBL

Instructions for NIH Clinical Center CRIS orders of Molecular Pathology

1. The Molecular Diagnostics Lab of the Laboratory of Pathology (LP), CCR, offers a menu of diagnostic tests that are used in the diagnosis and treatment of cancer, and immune disease-related disorders. These tests may be found in the CRIS under Anatomic Pathology orders.
2. To order a molecular diagnostic test offered by the Molecular Diagnostics Unit, LP, CCR, first enter the CRIS ordering system.
3. Select the **Anatomical Pathology** subsection.
4. Select **Molecular Pathology** test orders. Be aware that there are two categories of Molecular Pathology tests, “Molecular Pathology” (for all users) and “Molecular Pathology – Anatomical Path”. Do not select the latter; it is reserved for internal specimen processing by LP staff.
5. **CAREFULLY** select the appropriate test(s) that you wish performed. The molecular diagnostics laboratory printer only prints the tests you have selected. It is critical that you select the correct tests to avoid delays. If incorrect tests are ordered, you will have to submit a new CRIS order, although for most cases you will not need to submit an additional sample. We retain extracted DNA samples for a minimum of 10 years.
6. Please use the “special instructions” box as necessary.
7. Review your order and submit it in CRIS. The order will print out at the Molecular Pathology laboratory printer.
8. If you have any question regarding which test to order, please call the laboratory for advice. It will save time and effort in the long run!

Specimen and Handling Requirements

1. **Peripheral Blood:** 4 cc whole blood collected in light blue citrate tube and must put a second CRIS order (Research Blood) to Outpt Phlebotomy
2. **Bone Marrow Aspirates:** draw 2-3 cc in plain syringe with NO anticoagulant and immediately place into lavender top EDTA tube
3. **Paraffin-embedded tissue specimens** should be formalin fixed. **Either tissue blocks or unstained slides are acceptable.** The amount of tissue required for oncology tests on solid tumor is variable depending upon both specimen size and tumor content of the specimen. If you submit unstained slides, we request **10 unstained slides for small biopsies and cytology specimens**, or **5 unstained slides for larger excisional biopsies and resection specimens**. Generally 20% tumor cells within the specimen are required for most mutation tests.
4. When submitting the molecular pathology order for tests on formalin-fixed, paraffin-embedded (FFPE) tissue, there are several possible situations that dictate how the order and materials should

be submitted. These are listed below. Please follow the instructions appropriate for your patient's situation.

- **The case has been signed out by the NCI, Laboratory of Pathology (LP) and specimen was retained in LP.** In this case, a single molecular pathology CRIS order is required for subsequent molecular pathology tests. Please indicate the LP surgical or cytopathology case number in Special Instructions field of the request, if known (this number can be obtained from the NCI, LP, pathology report).
 - **A new tissue specimen is being submitted from a procedure performed at the NIH CRC.** In this case, two separate CRIS orders are required -- one for surgical pathology or cytopathology to request routine pathology services, and a second CRIS order for the molecular pathology. Please indicate in the special instructions box on the surgical pathology or cytopathology CRIS order that molecular testing is being requested on the sample.
 - **New submitted material from an outside hospital on a patient already admitted to the NIH CRC.** In this case bring the specimens (tumor block, or stained slides, unstained slides) with the outside pathology report to the Surgical Pathology or Cytopathology office, as appropriate. Two separate CRIS orders are required -- one for surgical pathology or cytopathology, and a second CRIS order for molecular pathology. Please indicate in the special instructions box on the surgical pathology or cytopathology CRIS order that molecular testing is being requested on the sample.
 - **New material from an outside hospital on a patient not yet admitted to the NIH CRC (CRIS ordering is not available).** In this case bring the specimens (tumor block, or stained slides, unstained slides) with the outside pathology report to the Surgical Pathology or Cytopathology office as appropriate, and fill out the hand-written requisition form for surgical/cytopathology examination and specify the molecular test required. The resident or fellow responsible for the case will submit the molecular requisition.
5. Due to CAP regulations, the laboratory personnel cannot provide the test results to physicians without permission. If you have an urgent case, please contact the Molecular Diagnostics Laboratory. Dr. Raffeld or another pathologist will discuss the results with you.

For more detailed specimen collection and handling requirement, please go web site:

<http://home.ccr.cancer.gov/LOP/Clinical/specimencollections.asp#molecular>

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