

IDENT	LabComp100.010
Type of Document	Policy
Applicability Type	Department-Level
Title of Owner	Network Dir Ops Lab&Path Svcs
Title of Approving Official	Medical Director of Clinical Laboratories
Date Effective	7/11/2014
Date of Next Review	7/30/2017



TITLE: Laboratory Reflex Testing Policy

PURPOSE: The purpose of this policy is to describe University of Vermont Medical Center (UVMC) Laboratory's Reflex Testing Practice.

POLICY STATEMENT:

University of Vermont Medical Center Laboratory offers reflex testing in accordance with the Office of Inspector General's Compliance Program Guidelines for Clinical Laboratories. It is the policy of University of Vermont Medical Center Laboratory to list tests subject to reflex on the laboratory requisitions and in our Laboratory Services Directory and to allow physicians the opportunity to decline the reflex testing if they believe it is not medically necessary. University of Vermont Medical Center Laboratory will perform reflex tests automatically when the following conditions are met:

1. Physician orders a test listed in Appendix 1 and:
2. The initial test result meets the criteria listed in Appendix 1 for prompting a reflex test or:
3. The specimen was sent to anatomic pathology and additional studies are needed to complete the evaluation of the case. Please see Anatomic Pathology and Reflex Testing (Appendix 2).
4. Bone marrow with reflex ordered.

The physician has the option of declining reflex testing. The decision to decline can be communicated to the laboratory via the laboratory requisition, in the Prism electronic ordering system or by contacting Laboratory Customer Service.

If a test subject to reflex testing is added by phone, the physician or their designee must communicate to the lab if they wish to decline the reflex testing otherwise the conditions for reflex as stated in this policy will apply.

All reflex testing is reviewed and approved by the laboratory pathologists or their designee on an annual basis. All UVMC Laboratory clients will be notified of changes to the reflex policy. University of Vermont Medical Center laboratory bills for the reflex tests it performs using the CPT code listed in the chart.

PROCEDURE:

1. Laboratory staff must note at time of order entry whether the physician has declined any reflex testing. If the lab order indicates the physician has declined a specific reflex then proceed to step 2.
2. Order the test and append message code **REFLEX** to order code. This appended message code will alert the technologist performing the test that the physician has declined the reflex portion. NOTE: Microbiology accessions their own specimens and will put in a message to indicate reflex declined at SREQ in Misys.
3. The technologist must make note that the physician has declined the additional reflex testing when they see the message code **REFLEX** attached to a test and complete steps 4 and 5.
4. The technologist will result the reflex test with the message code **REFLEX**.
5. The technologist will credit the reflex test using CRW function in Sunquest as necessary.

MONITORING PLAN: See Clinical Laboratory Services Auditing Practices

DEFINITIONS:

“Reflex Testing” is testing which is performed as a result of initial test results. Reflex tests are used to further identify significant diagnostic information required for appropriate patient care.

“Initial Test” is defined as a test subject to reflex testing in accordance with this policy (see Appendix 1).

The message code” REFLEX” decodes to *reflex testing declined by physician*.

RELATED POLICIES: Clinical Laboratory Services Auditing Practices

REFERENCES: OIG Compliance Program Guidance for Clinical Laboratories, August 1998.

REVIEWERS:

Kathy Nadeau, MLT(ASCP) (CPC), Laboratory Compliance Analyst
Carrie Stoner, MT (ASCP), Laboratory Compliance Analyst
Pamela Gibson, M.D, Division Chief, Anatomical Pathology
Cindy Cruickshank, MT(ASCP), Supervisor Specimen Receiving
Cindy Nelson, Manager, Clinical Laboratory Manager
Monica Sullivan, MT (ASCP) Clinical Laboratory Manager
Tim St. John, Manager, Anatomic Laboratory
Lynn Bryan, Manager, Business Systems and Client Services
Nicole Carney, Manager, Lab Quality
Michael Lewis, M.D., Division Chief, Pathology & Laboratory Medicine

OWNER: Tania Horton, Network Dir Ops Lab&Path Svcs

APPROVING OFFICIAL: Mark Fung, MD, Medical Director of Clinical Laboratories

UVMHC LABORATORY TESTS SUBJECT TO REFLEX

Initial Test	Reflex Criteria	Reflex Test(s)	Additional CPT billed
Anti-Actin IgG Antibody	Positive	SMA at Mayo	86255 86256 (if applicable)
Anti neutrophil Cytoplasmic Ab	Positive at screening dilution	Anti neutrophil Cytoplasmic Ab titer	86256
Anti neutrophil Cytoplasmic Ab	Positive perinuclear pattern and/or positive cytoplasmic pattern	Myeloperoxidase Ab (MYL) and PR3AB at Mayo	83516 x2
Antinuclear Ab	ANA positive at screening dilution	Antinuclear Ab titer	86039
Dilute Russell Viper Venom	Result above normal range	LA Confirm test	85613
Fetal Screen	Positive	Kleihauer Betke	85460
Fluid cell count	Any wbc's present	Differential	89051
Hemagram & Differential	See Lab service directory	Pathologist's smear review and interpretation	85060
Hemoglobin A1C	Suspicious Hgb not previously identified	HGB/Thalassemia Evaluation (HBEVAL)	83020/85660/83021
Hepatitis A Antibody	Positive result	Hepatitis A-IgM Antibody confirmation	86709
Hepatitis B Surface Antigen	Positive and Index Value <= 50	Hepatitis B Surface Ag Confirmation	87341
Hepatitis C Antibody(HCSCR)	Reactive	HCV RNA Detect Quant (HCVQU)* *with enough sample volume	87522
HCV RNA Quant w reflex to Genotype (HCVRX1)	>15 IU/ml	HCV genotyping (HCVGEN)	87902
HLA Class I Antibody screen	Positive	HLA Class I AB ID	86832
HLA Class II Antibody screen	Positive	HLA Class II AB ID	86833
Lipid Panel	Triglycerides > 400 mg/dl	Measured LDL	83721
Lyme Antibody	Pos or equivocal result	Lyme Western blot	86617 x2
Platelet Function Analysis	Above normal limit	COL/ADP cartridge	85576
Protein Electrophoresis, Serum	Suspicious band not previously identified	Immunotyping	86334
Protein Electrophoresis, Urine	Suspicious band not previously identified	Immunotyping	86335
Hemoglobin S Screen	Positive result	HGB/Thalassemia Evaluation (HBEVAL)	83020/85660/83021
Urinalysis w/reflex (UA)	When protein ≥1+	Urine microscopic	81001
Respiratory AFB Culture/Smear	1st time AFB smear positive(non-CF patients)	M. Tuberculosis complex, PCR	87556
Syphilis Serology : Treponemal Ab(IgG & IgM) Syphilis Ab (IgG) RPR	Reactive or Equivocal Reactive Nonreactive	Syphilis Ab (IgG @ Mayo) RPR/RPR titer OR Syphilis TP-PA serum	86780 86592/86593 86780

Appendix 1 con't

Note : The following lab order codes include a reflex scenario which is expressly stated as part of the order. In this case, there is no option to decline.

Initial Test	Reflex Criteria	Reflex Test(s)	Additional CPT billed
HGB/Thalassemia Evaluation (HBEVAL) (Capillary Electrophoresis performed 1 st)	Suspicious for abnormal Hgb	Acid plate electrophoresis	83020
	Suspicious for Hgb S	Acid plate electrophoresis and Hgb S screen	83020 and 85660
Thyroid Cascade (TTC)	TSH done 1 st . If outside normal range a free T4 is ordered. If free T4 is <1.9 ng/ml and TSH is <0.1 uIU/ml, a total T3 is ordered	Free T4 Total T3	84439 84480
Culture if urinalysis positive (CIFP)	Positive nitrite or leukocyte esterase or >5 wbc/hpf	Bacterial culture	87086
LA Cascade DRVV Silica Clotting Time	If either the DRVV or Silica clotting time are prolonged	Additional testing will be performed; See Lab Test Catalog for list- LACASC	85613 x3, 85732 x3 85520 85670
PTT 50/50 mix (PTT50)	PTT done 1 st , if PTT is abnormal and patient is on heparin-heparin neutralization is performed If heparin neutralization is abnormal, PTT 50/50 mix is performed.	Heparin neutralization PTT 50/50 mix	85730 85730 85525

Appendix 2

Anatomic pathology and reflex testing

In anatomic pathology, a specimen is sent to the laboratory with the intent that the pathologist will evaluate the specimen thoroughly enough to make a diagnosis. To this end the pathologist uses their medical judgment in ordering and interpreting additional studies on the material which they feel are necessary to fully evaluate the specimen. In this regard the pathologist is acting as a consultant in the care of the patient. The additional studies are charged only when deemed medically necessary by the pathologist. Such cases would include, but is not limited to, ordering special stains, decalcification of the tissue, immunoperoxidase stains, microbiology cultures on fresh tissue, flow cytometry on certain tumors or products of conception, and electron microscopy as indicated

Providers can also request additional studies on anatomic pathology cases, usually following discussion of the case with the pathologist.

There are some tests which are often useful for prognosis and diagnosis which are not yet considered routine or standard of care. For these tests, reflex testing procedures have been made so that if a specimen/process meets the reflex criteria then the additional testing will be performed.

Providers may decline the reflex testing by completing the appropriate box on the surgical pathology requisitions, in the Prism electronic ordering system or by contacting Laboratory Customer Service.

The department of Anatomical Pathology will automatically perform reflex testing as outlined below.

1. HercepTest (C-erb-B2 immunoperoxidase stain) Reflex Testing on Breast Cancers

Her2 is part of the epidermal growth factor family of receptors. This receptor has several synonyms including c-erb-B2, Her2/*neu*, and Her2. Over expression of Her2 receptors on invasive breast carcinoma is an adverse prognostic factor; more importantly, it is an invaluable predictive marker for response to Trastuzumab. In breast cancer, Her2 gene amplification and Her2 protein receptor over expression are highly correlated. Assays to detect either receptor over expression or gene amplification have been shown to stratify patient response to Trastuzumab and both assays are recommended by the College of American Pathologists (CAP) and the American Society of Clinical Oncology (ASCO) for this purpose. The ASCO/CAP guideline recommends all invasive breast carcinomas be tested for Her2 status.

The relationship between Her2 receptor over expression, gene amplification, and response to Trastuzumab therapy has become complex over time. There is general agreement that gene amplified tumors or receptor over expressing tumors should be treated; however, it is not clear whether non-correlating cases have identical responses to therapy as correlating cases. Approximately 5-15% of Her2 over expressing tumors will not exhibit gene amplification and 2-5% of Her2 IHC negative cases will exhibit gene amplification. In addition, tumors with polysomy of chromosome 17 or additional copies of the Her2 gene (duplication) that do not meet criteria for amplification are currently the subject of clinical trials to determine whether they respond to therapy. A more complete understanding of these relationships is obtained when both assay results are considered.

When Her2 testing is requested without specification, the Her2 receptor over expression IHC assay AND gene amplification ISH assay, on 1+ and 2+ IHC results will be performed. When Her2 testing by IHC is requested alone, only the immunohistochemical assay will be performed; however, 2+ (equivocal/borderline) results require the additional gene amplification assay according to ASCO/CAP guidelines. When only the IHC assay is specified, the pathologist interpreting a 2+ IHC assay will order the ISH gene amplification assay as a reflex test unless specifically declined by the ordering physician. Her2 testing is performed as a reflex test on all breast core biopsy or excision specimens with invasive breast adenocarcinoma that are processed and interpreted by the University of Vermont Medical Center Division of Anatomic Pathology.

2. Estrogen and Progesterone receptor testing on breast biopsies with ductal carcinoma in situ (DCIS)

Recent studies have shown a therapeutic benefit in treating patients with DCIS with tamoxifen. Allred et al. presented data at two meetings, which have shown that women with estrogen receptor (ER) positive DCIS treated with tamoxifen have significant reductions in the incidence of ipsilateral and contralateral breast cancer. Their subsequent data showed no benefit for tamoxifen therapy in women with ER negative DCIS. Performance of the PR assay provides additional information on the integrity of the ER receptor axis.

As a result of this new data, we now perform reflex testing on breast core biopsies with DCIS alone for estrogen and progesterone receptors.

Guidelines for the testing include:

- 1) all DCIS cases will be tested unless the reflex testing is declined by the provider ordering the test or there is insufficient tissue present for testing;
- 2) the reflex testing will be performed on the first incidence of DCIS (typically the core biopsy);
- 3) if invasive tumor is also present in the tissue being tested, the report will reflect the ER/PR results of the invasive tumor only unless the DCIS pattern is significantly different from the invasive tumor;
- 4) ER/PR testing will be repeated on a subsequent biopsy/ excision which contains an invasive tumor when the original biopsy showed only DCIS.

Allred DC, et al. Estrogen receptor status as a predicative marker of the effectiveness of tamoxifen in DCIS: Findings from NSABP Protocol B-24. (Abstract 80) Modern Pathol 2003;16:21A.

3. Reflex cytogenetic testing on adult renal tumors

Although histologic analysis is the main stay to the diagnosis of adult renal neoplasms, cytogenetic analysis can provide supportive diagnostic information in many cases. For this reason, FAHC has initiated sending portions of renal neoplasms for cytogenetic analysis when we receive a fresh resection specimen, which has enough tumor to sample for cytogenetics. Conventional renal cell carcinomas typically have loss of the genetic material in the long arm of chromosome 3 and chromophobe carcinomas are typically characterized by monosomy of multiple chromosomes and hypodiploidy. Papillary renal cell carcinomas, in contrast, often have a better prognosis than conventional renal cell carcinomas and typically have a distinct cytogenetic profile with trisomy of chromosomes 7, 16 and 17 as well as additional genetic abnormalities. The histomorphologic features of these tumors may overlap in certain cases and thus cytogenetic analysis is felt to be useful in many cases.

4. Reflex testing on fatty tumors

Fatty tumors similarly may be difficult at times to classify on morphologic basis alone and Cytogenetics on lesions greater than 5 cm can add additional diagnostic information. Solitary lipomas have been shown to have translocations involving 12q13-15 as well as chromosomal rearrangements of 13q or 6p21-33. Atypical lipomatous tumor/well differentiated liposarcomas, on the other hand, commonly show ring chromosomes and long marker chromosomes from 12q13-15. Dedifferentiated liposarcomas may have additional complex aberrations. Finding specific cytogenetic changes can therefore support the histologic findings.

5. Reflex cytogenetic testing on soft tissue and bone tumors.

In addition to the current reflex policy in surgical pathology for submitting Cytogenetics on renal tumors and fatty tumors, soft tissue tumors and bone tumors including certain pediatric tumors will routinely be submitted for Cytogenetics. More data has emerged on the utility of Cytogenetics as an adjunct to traditional diagnostic methods such as histologic examination with H&E stains as well as immunohistochemical stains. Cytogenetics is considered integral to the diagnosis of some soft tissue and bone tumors, especially in cases which pose histologic challenge. Soft tissue and bone tumors with well-known chromosomal translocations and gene rearrangements include Ewing sarcoma/PNET, desmoplastic small round cell tumor, extraskeletal myxoid chondrosarcoma, synovial sarcoma, alveolar rhabdomyosarcoma, low grade fibromyxoid sarcoma and inflammatory myofibroblastic tumor, to name a few.

6. Reflex mismatch repair protein (MLH1, PMS2, MSH2 and MSH6) immunohistochemical staining for screening patients with colorectal cancers for Lynch Syndrome

Lynch Syndrome (LS), also known as hereditary non-polyposis colorectal cancer (HNPCC), is the most common hereditary colon cancer syndrome, accounting for 3-6% of the total colorectal cancer burden. Microsatellite instability (MSI) due to germline mutation in one of the four DNA mismatch repair genes (e.g.: MLH1, MSH2, MSH6 or PMS2) is the hallmark of LS. According to the international criteria for LS diagnostics, cancer patients with a family history *or* early onset of colorectal tumors *or* tumors with specific MSI-High (MSI H) histology should receive genetic counseling and be offered testing for germline mutations in DNA repair genes. Recent data however shows that universal testing in all colorectal cancers yields higher detection rates and is a cost effective screening strategy. We therefore look at mismatch repair protein expression in colon cancer compared to the adjacent normal epithelium. This screening test will be performed on all newly diagnosed colorectal cancer; primarily in biopsy specimens and in resection specimens when biopsy material was performed elsewhere or when biopsy material gave equivocal results. Additional testing for MLH1 promoter methylation will be ordered upon receiving pre-authorization by the treating clinician if there is loss of MLH1/PMS2 protein expression and the test is clinically warranted.

REF:

1. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med.* 2005;352:1851-1860.
2. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol.* 2005;23:609-618.
3. Pérez-Carbonell L, Ruiz-Ponte C, Guarinos C, Alenda C, et al. Comparison between universal molecular screening for Lynch syndrome and revised Bethesda guidelines in a large population-based cohort of patients with colorectal cancer. *Gut.* 2011 Aug 25.
4. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med.* 2009 Jan;11(1):35-41.
5. Ladabaum et al., Strategies to Identify the Lynch Syndrome Among Patients With Colorectal Cancer: A Cost-Effectiveness Analysis. *Annals of Internal Medicine,* 2011; 155: 69-79

7. Reflex testing for MGMT promoter methylation on Glioblastomas

Glioblastomas are high grade malignant glial tumors of the brain. National Studies suggest that all Glioblastomas be tested for MGMT promoter methylation to help determine chemotherapy responsiveness and prognosis for patients with these neoplasms. MGMT (O (6) – methylguanine – DNA Methyltransferase) is a DNA repair enzyme that is involved in the repair of damage caused by a variety of DNA crosslinking compounds, including alkylating agents. Approximately 40 to 45% of Glioblastomas exhibit MGMT gene methylation. Retrospective studies have shown that detection of MGMT promoter methylation in tumor samples is associated with an increased likelihood of a favorable response to temozolomide (an alkylating agent, Temodar ®). (adapted from Mayo Medical Laboratory catalog).

Our Department will now be sending a representative block or unstained sections from patients with Glioblastomas to Mayo Medical Laboratories for MGMT promoter methylation testing. The results of the testing will be reported in an MGMT procedure report and will be available in PRISM.

8. Reflex testing 1p/19q Deletion in Gliomas

Testing for 1p/19q deletion is useful as an aid in diagnosing oligodendroglioma tumors and predicting the response of an oligodendroglioma to therapy. The test is indicated when a diagnosis of oligodendroglioma, both low-grade World Health organization (WHO, Grade II) and anaplastic (WHO, Grade III) is rendered. The test also is strongly recommended in mixed oligoastrocytomas.

Astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas are major histologic types of human gliomas; histologic differentiation among these tumors can be difficult. Recently, it has been shown that specific genetic alterations are highly associated with specific morphologic types of gliomas. (1) In addition, specific genetic alterations seem to predict prognosis (survival), as well as response to specific

chemotherapeutic and radio therapeutic regimens, irrespective of tumor morphology. (1-4)

Our laboratory will send a representative sample of the tumor to Mayo Medical Laboratories for 1p/19 q Deletion testing on brain tumors with oligodendroglioma features.

1. James CD, Smith JS, Jenkins RB: Genetic and molecular basis of primary central nervous system tumors. In Cancer in the Nervous System. Edited by VA Levine. New York, Oxford University Press, 2002, pp 239-251
2. Cairncross JG, Ueki K, Zlatescu MC, et al: Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst 1998 October 7;90(19):1473-1479
3. Ino Y, Zlatescu MC, Sasaki H, et al: Long survival and therapeutic responses in patients with histologically disparate high-grade gliomas demonstrating chromosome 1p loss. J Neurosurg 2000 June;92(6):983-990
4. Smith JS, Tachibana I, Passe SM, et al: PTEN mutation, EGFR amplification, and outcome in patients with anaplastic astrocytoma and glioblastoma multiforme. J Natl Cancer Inst 2001 August 15;93(16):1246-1256

9. Reflex HercepTest™ (HER2 immunoperoxidase stain) testing on advanced gastric and gastroesophageal junction cancers

BACKGROUND:

Despite ongoing advances in the treatment of gastroesophageal junctional (GEJ) and proximal gastric cancers the prognosis remains poor. The current guidelines recommend the use of molecular-based approaches to guide patient treatment options. Overexpression of the HER2neu protein, a member of the EGFR family, has been shown to play a strong role in the pathogenesis of several human cancers. The success of Trastuzumab (a targeted monoclonal antibody therapy also known as Herceptin) therapy in patients with HER2-positive (overexpressed or amplified) breast cancer has appropriately driven research in other cancers including gastric and GEJ tumors. The ToGA trial is the largest to date to address the use of Trastuzumab in combination with standard chemotherapy as an option for patients with HER2-positive advanced gastric or GEJ cancers. In the ToGA trial the addition of Trastuzumab to standard regimen chemotherapy resulted in a clinically and statistically significant benefit in terms of both median progression-free survival and median overall survival in Her2 positive patients. The current national comprehensive cancer network (NCCN) guidelines on assessment of overexpression of HER2-neu in GEJ and proximal gastric cancers are based on the criteria outlined in the ToGA trial.

REFLEX TESTING:

In compliance with the current NCCN guidelines, the division of surgical pathology will perform reflex HER2 testing in all patients with known (documented or clinically suspected) metastatic gastric or GEJ cancer.

Outline of scenarios for HER2 reflex testing

- 1) Any liver specimen or other organ that has a diagnosis of “metastatic adenocarcinoma, consistent with known gastric primary” will automatically get tested if formalin fixed paraffin embedded tissue is available on the metastasis.
- 2) If gastric/GEJ biopsy being performed on primary mass and clinician is aware of metastatic disease, this must be indicated on requisition “gastric/GEJ tumor with M1 disease or metastatic disease”.

TEST ALGORITHM:

HercepTest™ (Dako Corp) is an FDA-approved standardized immunohistochemical assay that measures HER2 protein overexpression in tumors and was the assay used in the ToGA trial. The HercepTest™ is the first line test on all metastatic gastric and GEJ cancers. It is scored as 0-3+ with 0 and 1+ considered a negative result for protein overexpression and 3+ considered as positive for protein overexpression. A 2+ result is considered equivocal and in keeping with the current NCCN guidelines, 2+ tumors are automatically referred for fluorescence in situ hybridization (FISH) to evaluate for HER2 gene amplification in the tumor.

References:

Di Fiore F, Blanchard F, et al. Clinical Relevance of KRAS Mutation Detection in Metastatic Colorectal

Cancer Treated by Cetuximab Plus Chemotherapy.
BJCancer (2007) 96: 1166-1169

Amado G, Wolf M, et al. Wild-Type KRAS is Required for Panitumumab Efficacy in Patients With Metastatic Colorectal Cancer.
J Clin Oncol 2008, apr 1; 26(10): 1626-1634

Allegra C, Jessup M et al. American Society of Clinical Oncology Provisional Clinical Opinion: Testing for KRAS Gene Mutations in Patients with Metastatic Colorectal Carcinoma to Predict Response to Anti-Epidermal growth Factor Receptor Monoclonal Antibody Therapy.
J Clin Oncol, Vol 27, No 12, 2009: 2091-2096

Astrid L, Jean Baptist B, et al. KRAS Mutation Status Is Predictive of Response to Cetuximab Therapy in Colorectal Cancer.
Cancer Res 2006; 66: (8): 3992-3995

10. Reflex BRAF V600E mutational analysis for melanoma

BRAF inhibitors are drugs designed to target a somatic mutation in the BRAF gene and have been approved by the FDA for the treatment of metastatic melanoma or unresectable primary melanoma. BRAF codes for a kinase component in the RAF-MEK-ERK (MAPK) signal transduction phosphorylation cascade. Mutations in the BRAF kinase gene are common in tumors of patients with advanced melanoma and result in constitutive activation of the MAPK pathway which is believed to be actively involved in oncogenic proliferation. Direct and specific inhibition of the mutated kinase has been shown to significantly retard tumor growth and may improve patient survival. In general, 50-70% of melanoma tumors harbor a BRAF mutation; of these, 80% are positive for BRAF V600E mutation and 16% are positive for BRAF V600K mutation. Thus, approximately 45-60% of advanced melanoma patients may respond to a BRAF inhibitor targeted to this mutated kinase.

Vemurafenib (Zelboraf®) is an oral selective BRAF inhibitor that is approved by the FDA for patients with advanced melanoma and BRAF V600E mutation confirmed by an FDA approved test. Preclinical studies showed paradoxical acceleration of growth in melanoma tumors with the BRAF wild type gene sequence, suggesting that it might be harmful to administer BRAF inhibitors to patients with BRAF wild type melanoma. The approval of vemurafenib was supported by an international, multicenter trial that screened 2,107 patients with previously untreated, stage IIIC or IV melanoma for the BRAF V600 mutation and identified 675 patients by the cobas® 4800 BRAF V600 Mutation Test (1). Patients were randomly assigned to receive either vemurafenib or dacarbazine. Vemurafenib produced improved rates of overall and progression-free survival. Vemurafenib was also shown to induce clinical responses in more than half of patients with previously treated BRAF V600-mutant metastatic melanoma in a multicenter phase II trial (2).

Currently, testing is performed at Mayo Medical Laboratories using a PCR-based assay that targets the BRAF V600E mutation with BRAF wild-type and V600E target specific fluorescent dye-labeled TaqMan probes. A formalin-fixed, paraffin-embedded (FFPE) tissue block is the preferred specimen, although 1 slide stained with hematoxylin and eosin and 5 unstained, nonbaked slides (5-microns thick sections) of the tumor tissue may also be supplied for testing. FAHC will provide reflex testing for BRAF V600E mutation on all new specimens of metastatic melanoma (Stage IIIC and IV) or unresectable primary melanoma. Testing of a primary lesion may be considered if there is difficulty obtaining sufficient tissue sample in a patient with metastatic disease. The results of the mutational analysis will be reported in a BRAF mutation procedure report and will be available on PRISM.

References:

1. Chapman PB, Hauschild A, Robert C, et al.: Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 364 (26): 2507-16, 2011.

Printed on: 6/8/2017 3:39 PM By: %username%

DISCLAIMER: Only the online policy is considered official. Please compare with on-line document for accuracy.

Sosman JA, Kim KB, Schuchter L, et al.: Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med* 366 (8): 707-14, 2012.

11. Afirma Gene Expression Classifier (GEC)

This test is utilized in the management of patients with Thyroid Fine Needle Aspirations demonstrating an indeterminate thyroid nodule.

Clinical Application:

Approximately 525,000 thyroid nodule Fine Needle Aspirations (FNA) were performed in the United States in 2011.¹ Cytopathology FNA samples can be challenging to interpret. Indeterminate results are generated in 15-30% of the cases.² Prior to Afirma testing, guidelines recommended that most of these patients undergo surgery to assess whether the nodules were benign or malignant.³ In approximately 70-80% of the cases the nodules were determined to be benign by surgical pathology.⁴ The Afirma GEC reduces the number of surgeries by identifying indeterminate Cytopathology Thyroid FNAs which are low risk for cancer.

Afirma Gene Expression Classifier:

The Afirma GEC measures the expression of 142 genes to determine if the thyroid nodule FNA sample is benign or suspicious for cancer. Patients with benign Afirma results can potentially avoid unnecessary surgery. Results will be available in CoPath and in PRISM. Clinical usage of the GEC is supported by clinical practice guidelines such as the American Thyroid Association.⁵

Reflex Parameters:

If the cytopathology diagnosis is indeterminate (Bethesda Category III-IV) which includes Follicular Lesion of Undetermined Significance, Atypia of Undetermined Significance, Follicular Neoplasm/Suspicious for Follicular Neoplasm and Hürthle Cell Neoplasm/Suspicious for Hürthle Cell Neoplasm, the thyroid nodule is greater than or equal to 1 cm and the patient is 21 years or older, the Afirma Gene Expression Classifier is performed.

Note: The presence of oncocytic (Hürthle cell) features can trigger a Suspicious Afirma result. Therefore the Positive Predictive Value is lower in these cases.⁶

References:

1. Sosa JA, Hanna J, Lanman RB, Robinson KA, Ladenson PW. Increases in thyroid nodule fine needle aspirations, surgeries and diagnosis of thyroid cancer in the United States, American Association of Endocrine Surgeons 34th Annual Meeting, Apr 14-16, 2013, Chicago, IL. (oral abstract).
2. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* 2009;19:1167-1214.
3. Gharib H, Papini E, Paschke R, Duick DS, Valcavi R, Hegedus L. et al. American Association of Clinical Endocrinologists, Associazione Medicie Endocrinologi, and European Thyroid Association medical guidelines for clinical practice for the diagnosis and management of thyroid nodules: executive summary of recommendations. *Endocr Pract.* 2010;16(S1): 1-43.
4. Wang CC, Friedman L, Kennedy GC, Wang H, Kebebew E, Steward DL, et al. A large multicenter correlation study of thyroid nodules; cytopathology and histopathology. *Thyroid.* 2011;21:249-251.
5. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid.* 2016;26:1-133.
6. Lastra RR, Pramick MR, Crammer JC, et al. Implications of a Suspicious Afirma Test Result in Thyroid Fine-Needle Aspiration Cytology: An Institutional Experience. *Cancer Cytopathology.* 2014;122:737-744

12. Reflex genomic testing on metastatic colorectal tumors:

Evidence supports mutational testing for EGFR signaling pathway genes, since they provide clinically actionable information as negative predictors of benefit to targeted therapies for colorectal cancer. Mutations in several of the biomarkers have proven prognostic value. For these reasons, the transdisciplinary clinical team at UVMC that manages cancers of the gastrointestinal tract have decided it is best clinical practice to reflexively submit tumor tissue from samples diagnosed as “metastatic colorectal cancer” for targeted genomic profiling in the Genomic Medicine Laboratory. The assay, GenePanel Solid Tumor, is analytically and clinically validated to detect somatic single nucleotide variants and insertion/deletion mutations in all of the genes considered “clinically-relevant” for managing this cancer type, specifically “expanded RAS testing”. This policy does not apply to slide-only consultations and tissue procured in New York State.

13. Reflex Testing for Hematolymphoid Malignancies

Advances in understanding and treatment of lymphomas, leukemias, and other hematolymphoid malignancies have accelerated the use of advanced laboratory techniques to diagnose and classify these diseases, as well as to generate prognostic and predictive information that facilitates personalized care for our patients. Often the need for a particular test only becomes apparent after initial assessment using standard approaches such as microscopy and immunohistochemistry.

At the time of biopsy, it can be difficult to anticipate the array of tests that may be needed to complete a patient’s workup; additionally, some samples may be difficult or impossible to collect a second time. Furthermore, when a physician’s intent is to establish a diagnosis to facilitate patient care, he or she may reasonably expect colleagues in the clinical laboratory to apply their expertise and employ techniques appropriate to a particular case.

To facilitate timely performance of additional testing in the setting of hematolymphoid disease, reflex testing will be available to physicians who suspect that a patient has a hematolymphoid malignancy and order tests such as bone marrow examination, flow cytometry, and/or cytogenetics. As always, the option to decline such testing will be available. If reflex testing is not declined, then one of UVM Medical Center’s hematopathologists may order additional tests that are medically indicated to complete the assessment of a patient’s blood, bone marrow, lymph node, or other specimen. Our hematopathologists will continue to work closely with colleagues in hematology/oncology and other fields to ensure that testing is appropriate for particular patients, that appropriate samples are collected, and that the array of available tests meets the rapidly evolving requirements for the complex management of these diseases.

Examples of reflex testing for Hematolymphoid Malignancies:

- Cytogenetic testing of a bone marrow sample is indicated when a patient has cytopenias and myelodysplastic syndrome is in the differential diagnosis.
- FISH for t(11;14) can assist in ruling out mantle cell lymphoma when flow cytometric analysis of a fine needle aspirate indicates lymphoma but yields non-specific findings with respect to classification.
- BCR/ABL testing by FISH or PCR can assist when flow cytometry shows marked granulocytic hyperplasia and there is clinical suspicion of a myeloproliferative neoplasm.
- Mutational analysis of FLT3, NPM1, CEBPA, and/or other genes in cases of acute myeloid leukemia yields important prognostic information.
- FISH for rearrangements of MYC, BCL2, and/or other genes can yield results that influence therapeutic decision-making in cases of aggressive B-cell lymphomas.
- T-cell receptor and/or IGH gene rearrangement studies may be helpful when the distinction between lymphoid hyperplasia and lymphoma is not straightforward on the basis of morphology and immunohistochemistry.

References:

Swerdlow SH, et al, eds. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC Press, 2008.

Swerdlow SH, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood 2016;127:2375-2390.

Arber DA, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 2016;127:2391-2405.

National Comprehensive Cancer Network. NCCN guidelines for treatment of cancer by site. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#site. Accessed 24 April 2017.

Initial Test	Reflex Criteria	Reflex Test(s)	Additional CPT billed
Biopsy of metastatic focus	Stage 3C or 4 Melanoma or unresectable primary melanoma	BRAF V600E Mutation Analysis	81210
Breast excision for carcinoma	All invasive adenocarcinomas	HercepTest by IHC and CISH (If IHC result 1+ or 2+)	88360 88368
Breast biopsy	1 st incidence of DCIS	ER/PR receptor testing	88360 88360.26
Renal or Bone or Soft Tumor Excision	Enough tumor to sample	Cytogenetics	88233 88264 88291
Fatty Tumor excision	>5cm	Cytogenetics	88233 88264 88291
Colon or Rectal Biopsy	All invasive colorectal adenocarcinomas	Immunohistochemical testing (MLH1,PMS2,MSH2,MSH6) reflex to MLH1 Promoter methylation if loss of MLH1/PMS2 (with preauthorization from treating clinician) (performed at ARUP)	IHC-88342 x4 Methylation 81479
Colon Cancer resection	#1. ALL cases of colorectal cancer with inconclusive biopsy testing results or no prior testing on biopsy specimens. #2. Loss of MLH1/PMS2 on IPEX	#1. Immunohistochemical testing (MLH1,PMS2,MSH2,MSH6) performed at FAHC Lab If #2. MLH1 promoter methylation upon pre-authorization (performed at ARUP)	88342 x4 for MMR 81479
Brain Tumor Biopsy	Glioblastoma Diagnosis	MGMT Promoter Methylation	81479
Brain Tumor Biopsy	Dx of brain tumor w/oligodendroglioma features	1p/19q Deletion in Gliomas, FISH (@ Mayo)	88291 88275x2 88271x4
Gastric/GEJ bx with h/o of met OR Liver or other organ with dx of mets c/w known gastric or GEJ primary	Diagnosis of metastatic adenocarcinoma consistent w/ known gastric primary tumor OR gastric/GEJ biopsy being with M1 or metastatic disease	HercepTest; If Herceptest yields 2+ (indeterminate)result, FISH will be performed (Mayo clinic).	88360; If FISH performed 88271 x2, 88274, 88291.
Thyroid FNA	<ul style="list-style-type: none"> Atypia of Undetermined Significance Follicular Lesion of Undetermined Significance Follicular Neoplasm/Suspicious for Follicular Neoplasm Hürthle Cell Neoplasm/Suspicious for Hürthle Cell Neoplasm 	Afirma® GEC (Gene Expression Classifier)	81545-billed by Veracyte Inc

Microscopic analysis of anatomic pathology specimen	Microscopic Diagnosis of Metastatic Colorectal Cancer	GenePanel Solid Tumor	81445
Bone marrow aspiration and/or biopsy	Suspicion of a hematolymphoid malignancy	Cytogenetics, flow cytometry, FISH, PCR, mutational analysis, and/or genomic testing	Examples include 88233, 88264, 88291, 88184-88189, 81245, 81246, 81218, and/or other codes as may be applicable