Reflex Testing

Corewell Health South Lakeland

Contents

*Under certain circumstances reflex testing may be initiated based on NCCN guidelines, see MEC form (07/13/2020) for more details.

- Brain Tumors-
  - All WHO grade III (Anaplastic Astrocytomas) and grade IV (Glioblastoma) astrocytomas will have MGMT gene methylation testing.
  - All glial tumors grade II/III will have 1p/19q co-deletion status tested.
  - *IDH1/IDH2* mutations will be performed on all gliomas (grade II/III/IV).

- Breast carcinoma-

-All invasive breast carcinomas will have Ki-67 immunohistochemistry performed at the time of diagnosis along with ER, PR, and HER2/neu.

Note: For specimens fixed >72 hours for Her2, ER & PR in which negative test results are obtained, the report should state that prolonged fixation could be a possible cause for the negative result, and alternative testing methods should be considered (e.g. FISH for HER2; gene expression assay for ER). For HER2 testing, labs should also consider confirming by FISH any specimen fixed longer than 72 hours that is not score 3 by IHC.

-All invasive breast carcinomas will be tested for Her-2/neu by immunohistochemistry and/or CISH or FISH.

- A new HER2 test should be ordered if the initial HER2 test is positive in a histologic grade 1 infiltrating ductal or lobular carcinoma and the tumor is: a) hormone receptor-positive or b) 90 percent pure: mucinous, tubular, cribriform, or adenoid cystic histology.
- A new HER2 test may be ordered if the initial HER2 test is negative and: a) The tumor is Grade 3, b) the invasive component in the biopsy specimen was small, or c) the definitive resection contains a high-grade carcinoma morphologically distinct from that in the original biopsy. Updated ASCO/CAP guidelines no longer require repeat HER2 testing in grade 3 tumors.
- Repeat HER2 testing may be performed on the resected tumor if the core biopsy result was equivocal by IHC and ISH required recounting (groups 2-4). Updated ASCO/CAP guidelines for HER2 FISH require additional recounting on specimens formerly called "equivocal" to provide a final positive or negative status. Repeat testing on resection specimens in these cases (groups 2-4) is no longer required.
- ER/PR and HER2 testing should be performed at relapse when tissue from relapse biopsy is available. This includes *all* metastasis, irregardless of time course or if prior metastases have been tested.

-The surgeon will order an Oncotype DX if the tumor is: greater than 5mm, invasive ductal carcinoma, ER+, PR+ or PR-, HER2 negative and lymph nodes are negative or 1 to 3 LN positive. Oncotype should be sequential in bilateral breast cancers- start with aggressive tumor first, if it is negative/low risk send the other side. Send cores preferably; second choice is tumor without DCIS or nearby reactive change.

In all other situations including favorable histologies (e.g. tubular or mucinous), classical invasive lobular carcinoma, poor histologic differentiation or lymph node micrometastasis the medical oncologist is responsible for ordering the Oncotype DX. The treating oncologist may order a

MammaPrint in place of Oncotype DX. Send cores preferably; second choice is tumor without DCIS or nearby reactive change.

- ## Colorectal cancer-

MMR by immunohistochemistry.

- MMR IHC on all tumors (use biopsy if possible). If MLH1 and PMS2 loss, send for MLH1 hypermethylation and BRAF analysis
- If no MMR testing is performed (limited tumor volume on biopsy, resection specimen where testing already performed) include a comment explaining why testing is not done (for billing compliance).
- **Rectal tumor**: Make every effort to perform MMR IHC on rectal biopsies, these patients often get neoadjuvant therapy and may not go to resection or may have no residual tumor.

Stage 4: In addition to MMR IHC (as above) perform expanded Next Generation sequencing if enough tissue, otherwise targeted colon panel and HER2/neu by immunohistochemistry.

Use the "Colon and Rectum: Biomarker Reporting Template" in Epic Beaker.

-These tumors will be scored using the breast scoring criteria.

-This will be classified as FDA cleared test (DAB/Ventana).

-Primary antibody is PATHWAY (synonymous with 4B5).

- ## Lung cancer (Non-small cell carcinoma)-

Lung Cancer Staging

-Stage 4:

Next generation sequencing, PD-L1, and MMR immunohistochemistry

-Clinical stage 1b or greater:

- Clinical stage 1b or greater: any tumor greater than 3 cm or tumor of any size with positive lymph node or suspicion of positive lymph nodes
- To be performed at time of biopsy if possible, otherwise perform on resection
- NGS and PD-L1

-Tumor 3-4 cm in size without evidence of lymph node involvement:

- To be performed at time of biopsy if possible, otherwise perform on resection
- NGS

- ## Small intestine adenocarcinoma-

- All adenocarcinomas of small intestine (including ampullary) will have MMR by immunohistochemistry.

- ## Gastrointestinal stromal tumor-

  - All gastrointestinal stromal tumors with high-risk features will be reflexively sent for next generation sequencing.

- ## Gastroesophageal adenocarcinoma-

  - New gastroesophageal adenocarcinomas will have MMR by immunohistochemistry and HER2/neu by immunohistochemistry run.
  - All metastatic gastroesophageal adenocarcinomas will be tested for PD-L1 and MMR by immunohistochemistry.

Table 1: HER2 testing for gastric and GEJ adenocarcinomas (Histopathology 2008;52:797, Arch Pathol Lab Med 2016;140:1345)

Pattern of HER2 membrane reactivity by IHC in surgical specimen	Pattern of HER2 membrane reactivity by IHC in biopsy specimen	HER2 score by IHC	HER2 status by IHC	Recommendations for ISH testing and treatment
No reactivity or membrane reactivity in < 10% of tumor cells	No membrane reactivity in cancer cells	0	Negative	No ISH testing HER2 targeted treatment not recommended
No more than faint partial membrane reactivity in 10% or more of tumor cells	A cluster of at least 5 cancer cells with no more than faint membrane reactivity regardless of the percentage of positive cancer cells	1+	Negative	No ISH testing HER2 targeted treatment not recommended
10% or more of the tumor cells show weak to moderate lateral or complete basolateral membrane reactivity	A cluster of at least 5 cancer cells with weak to moderate lateral or complete basolateral membrane reactivity regardless of the percentage of positive cancer cells	2+	Equivocal	Reflex to ISH Administer HER2 targeted treatment only if ISH positive
10% or more of the tumor cells show strong lateral or complete basolateral membrane reactivity	A cluster of at least 5 cancer cells with strong lateral or complete basolateral membrane reactivity regardless of the percentage of positive cancer cells	3+	Positive	No ISH testing Eligible for HER2 targeted treatment

-Use the "Gastric HER2: Biomarker Reporting Template" in Epic Beaker.

-These tumors will be scored using the gastric scoring criteria (see attached table and paper for criteria and additional information on scoring).

-This will be classified as FDA cleared test (Ultraview DAB/Ventana).

-Primary antibody is 4B5

- ## Gynecologic Malignancy-

  General reporting request: report the presence or absence of extranodal extension for any malignancy type (even if not required in the CAP checklist).

  - Uterine carcinoma:
    - MMR IHC on all tumors (use biopsy if possible). If MLH1 and PMS2 loss, then send for MLH1 hypermethylation analysis
    - P53 IHC on all tumors (use biopsy if possible).
    - POLE Testing if high risk features are present (non-endometrioid histology, p53abn, MMRd, Grade 3 histology, any LVSI, Deep myometrial invasion)
    - Her2 IHC if uterine serous carcinoma or carcinosarcoma with serous epithelial component (breast interpretation methods) reflex to FISH if necessary.

Note: grading Her2 depends on if they are using regular Trastuzumab vs Trastuzumab-deruxtecan (a HER2 and chemo drug conjugate), table per Anne Mills CAP lecture Oct 2024

Uterine Serous vs. Gastroesophageal Soring Systems

HER2 IHC Score	Uterine Serous Carcinoma Scoring (Buza et al.)	Gastroesophageal Scoring (Bartley et al 2016)
0	No staining in tumor cells	Resection: No reactivity or membranous reactivity in <10% of cells (resection) Biopsy: No reactivity in any tumor cells
1+	Faint/barely perceptible, incomplete membrane staining in any proportion, or weak complete in <10% of tumor cells	Resection: Faint/barley perceptible membranous reactivity in ≥10% of tumor cells; cells reactive in only part of their membrane Biopsy: Tumor cell cluster (≥ 5 cells) with faint or barely perceptible membranous reactivity irrespective of tumor cells stained
2+	Intense complete or basolateral/lateral membrane staining in ≤30%, or weak to moderate in ≥10% of tumor cells	Resection: Weak to moderate, complete basolateral or lateral membranous reactivity in ≥10% of tumor cells Biopsy: Tumor cell cluster (≥ 5 cells) with weak to moderate complete basolateral or lateral membranous activity irrespective of percentage of tumor cells stained.
3+	Intense complete or basolateral/lateral membrane staining in >30% of tumor cells	Resection: Strong, complete basolateral or lateral membranous reactivity in in ≥10% of tumor cells Biopsy: Tumor cell cluster (≥ 5 cells) with strong, complete basolateral or lateral membranous activity irrespective of percentage of tumor cells stained.

Buza N. Her2 Testing in Endometrial Serous Carcinoma: Time for Standardized Pathology Practice to Meet Clinical Demand. *Arch Pathol Lab Med* 2021;145(6):687-691.

Bartley et al. Her2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline from the American College of Pathologists, American Society for Clinical Pathology, and American Society for Clinical Oncology. *Arch Pathol Lab Med* 2016; 140(12):1345-1363.

(Tras only uses the Serous carcinoma scoring while the drug conj. Uses the Gastric (Because that trial did not involve pathologists):

- Ovarian clear cell, endometrioid, and mucinous carcinoma: MMR IHC. If MLH1 and PMS2 loss, then send for MLH1 hypermethylation analysis
- Ovarian low grade carcinomas (not benign or borderline): ER and PR testing.
- Ovarian high-grade serous carcinomas: HRD testing will be requested by the oncologists.
- Vulvectomy reporting requests: report out distance to margin for invasive and HGSIL, size of tumor metastasis and presence or absence of extranodal extension.

- ## Head and neck squamous cell carcinoma-

  - All oropharyngeal squamous cell carcinomas and/or regional lymph node metastases from an occult primary will be reflexively assessed for HPV by molecular testing or by immunohistochemistry for p16.

- ## Melanoma-

  - Metastatic melanomas will be reflexively sent for next generation sequencing. This includes sentinel lymph node metastases greater than or equal to 1 mm.
  - Melanoma local recurrence will be reflexively sent for next generation sequencing.

- ## Muscle Tumors-

Rhabdomyosarcoma: Cytogenetics on all tumors (PAC-FOX1 fusion, t(2;13) or t(1;13). Soft tissue sarcomas: Cytogenetics on all tumors.

- ## Pancreatic adenocarcinoma-

  - Metastatic pancreatic adenocarcinomas will have MMR by immunohistochemistry and HER2/neu by immunohistochemistry.
  Please use the "Quantitative IHC Biomarker Reporting Template" in Epic Beaker.

  -These tumors will be scored using the gastric scoring criteria (see attached table and paper for criteria and additional information on scoring).

  -This will be classified as FDA cleared test (Ultraview DAB/Ventana).

  -Primary antibody is 4B5.

- ## Prostatic adenocarcinoma-

  MMR testing by immunohistochemistry will be reflexively performed on metastasis.

- ## Solid Tumors-

  All the above solid tumors that have progressed following prior treatment and who have no satisfactory alternative standard treatment options or options through available clinical trials should have microsatellite instability testing performed.

  If the patient's tumor has not been tested previously for microsatellite instability or for PD-1 or PD-L1 or Tumor mutational burden, it should be performed at the request of the oncologist.

- ## Thyroid Neoplasms

  Related information is accounted for in the Cytopathology section.

- ## Unknown Primary

  For patients in whom the combination of a full IHC workup and thorough clinical and radiographic correlation fails to determine tumor lineage in the opinion of both the clinician and pathologist, molecular testing to assist in lineage assignment will be performed upon the request of the oncologist.

- ## Urothelial carcinoma, upper tract only

  - Testing for MMR by immunohistochemistry will be performed on all upper tract urothelial carcinoma cancer resections.
  - If loss of expression of MLH1 and PMS2 are evident by IHC, reflex testing for hypermethylation will occur.

Chemistry Reflex Testing

Chemistry – Mandatory	
Initial Test and Result	**Confirmation Testing/Additional Workup**
HIV Quick Test (LAB175) performed at Corewell Health Reference Laboratory West	If Reactive, reflex to HIV 1/HIV 2 Ab Ag Diagnostic (Roche) to be performed at Corewell Health Reference Laboratory West.
HIV 1/HIV 2 Ab Ag Diagnostic (Roche)	If Reactive, reflex to Geenius HIV 1/HIV 2 Antibody Confirmation
Reactive Hepatitis B surface antigen	HbsAg Confirmation test
Reactive Syphilis IgG Antibody	Reflex to RPR titer at Corewell Health West. If RPR is negative, additional TP-PA testing will be performed by MDHHS
Reactive Hepatitis C Virus Antibody	HCV RNA
Chemistry – Optional	
Initial Test & Result	**Optional Follow up Testing**
Mononucleosis Screen, Epstein Barr (EBV) IgM if Negative	EBV IgM
Lipid Panel do LDL Direct if Triglycerides >400 Triglyceride result > 400 mg/dL	LDL Direct
Thyroid Function Cascade TSH result above 5.0 mcU/mL **or** TSH result below 0.3 mcU/mL **or** TSH result below 0.1 mcU/mL and FT4 result below 1.6 ng/dL	FT4 and TPO if TSH is above 5.0 mcU/mL FT4 if TSH is below 0.3 mcU/mL • Free T3 if TSH is below 0.1 mcU/mL and FT4 is below 1.6 ng/dL
TSH, Free T4 if indicated TSH result above 5.0 mcU/mL **or** TSH result below 0.3 mcU/mL	Free T4

Coagulation Reflex Testing

Coagulation – Mandatory	
Initial Test and Result	**Confirmation Testing/Additional Workup**
aPTT with no endpoint detected or error code on the coagulation analyzer that cannot be resolved.	Unfractionated Heparin
Platelet Function Assay (PFA 100) with Collagen/Epinephrine cartridge results greater than 180 seconds.	Collagen/ADP test
Platelet Function Assay (PFA 100)	Platelet Count and hematocrit

Cytology Reflex Testing

Cytology - Mandatory	
Initial Test and Result	**Confirmation Testing/Additional Workup**
Gynecologic and Reproductive Malignancy	Related information included in the AP section
Pap and HPV testing	Reflex Information Is HPV Requested? a. If NO HPV testing is desired, select NO and only the pap test will be ordered. b. If YES, HPV testing is desired, select one of two options: 1. CO-TESTING (30-64 y/o) -If Co-testing is selected, the HPV test will be ordered and performed regardless of the pap test final diagnosis. Note: Co-testing is recommended for patients age 30-64. 2. HPV REFLEX (see link below for criteria) -If reflex is selected the HPV test will only be performed in the following scenarios: a. The pap test final diagnosis is ASCUS and the patient is between ages 21-64. b. The pap test final diagnosis is LSIL and the patient is between ages 25-64
Thyroid cases finalized with a Bethesda category of I, II, and VI, (Non-diagnostic, Benign, and Malignant) will not be sent for molecular testing. Cases finalized with a Bethesda category of III – V, (AUS/FLUS, Follicular Neoplasm or Suspicious for Follicular Neoplasm, Suspicious for Malignancy) will be reflexed for testing. Bethesda System for Reporting Thyroid Cytopathology 3rd ed. 2023- updated 2/2025 Per NCCN guidelines version 5.2024	Patients 21 years of age and older: - Afirma Genomic Sequencing Classifier (GSC) and Malignancy Classifiers (BRAF, MTC, RET/PTC1 and RET/PTC3); to Veracyte (fresh sample in Afirma vial) Patients under 21 years of age: - ThyroSeq Genomic Classifier testing to CBL Path (fresh sample in ThyroSeq vial, FFPE cell block, or smeared slide)

Cytology– Optional	
Initial Test and Result	**Optional Follow up Testing**
Cervical Cytology with ASCUS, ASC-H OR LSIL	HPV-high risk
Cervical Cytology with ASCUS or AGUS	HPV
Cervical Cytology with NIL, ASCUS or AGUS	HPV

Flow Cytometry Reflex Testing

Flow Cytometry – Mandatory	
Initial Test and Result	**Confirmation Testing/Additional Workup**
Diagnostic sample of B-lymphoblastic leukemia (B-ALL) and B-cell non-Hodgkin lymphoma with anti CD-19 therapy	Flow cytometry Blinatumomab tube (anti-CD19 therapy tubes)
Flow cytometry testing requiring CBC w diff for quantitation of flow cytometry results (i.e. SCID, ALPS, lymph subsets, CD20, etc)	1. CBC w diff on all orders that do not already have a CBC w diff ordered on patient on the same date and specimen is less than 10 hours old. 2. And Flow cytometry is unable to get WBC and automated differential.
Leukemia/Lymphoma/Myeloma and/or Non-Hodgkin lymphoma panels by flow cytometry: if indicated, reflex testing may be added to further characterize possible abnormal cell populations identified by the screening panel. These panels are reviewed continuously in multidisciplinary conferences and by the flow cytometry laboratory and hematopathologists.	The following add on panels may be employed after initial testing, as needed and appropriate, to further evaluate any possible abnormal population of cells. B lymphoblastic leukemia (B-ALL) panel T lymphoblastic leukemia (T-ALL) panel Chronic lymphocytic leukemia (CLL) panel Hairy cell leukemia (HCL) panel Extended B-cell tube panel Extended T-cell tube panel NK cell or LGL panel CD10 positive B-cell panel CD5 positive B-cell panel Acute myeloid leukemia (AML) panel Extended myeloid or monocytic panel Plasma cell panel Mast cell panel
Flow Cytometry – Optional	
Initial Test and Result	**Optional Follow up Testing**
Fetal Cells by Flow Cytometry → If ordered STAT and received in lab outside of flow cytometry testing hours (after 3:30 Mon-Fri or after 10:00am Sat or Sun)	Fetal Hemoglobin by Kleihauer Betke performed in place of flow cytometry
Leukemia or Non-Hodgkin Lymphoma Panel by Flow Cytometry → Cell population is diagnostic of circulating leukemia/lymphoma/myeloma, and patients under age of 80 with new diagnosis	FISH testing

Hematology Reflex Testing

Hematology – Mandatory	
Initial Test and Result	**Confirmation Testing/Additional Workup**
CBC w/ diff - will reflex to CBC w/out diff "if" the WBC is less than or equal to 0.4. Updated by Lab due to OEE March 2024	CBC w/out differential
Cerebral Spinal Fluid (CSF) RBC Cell Count greater than or equal to 400 cells in tube 3 Update Approved by MEC May 2022	Additional count of tube 1
Cerebral Spinal Fluid (CSF) WBC Cell Count greater than 0, in tube 3	Manual differential
Mononucleosis Screen, Epstein Barr (EBV) IgM if Negative: If Mononucleosis Screen is Negative	Epstein Barr (EBV) VCA IgM Acute Antibody
Pathologist Review If review of peripheral blood smear is ordered without required accompanying CBC with differential, and if the specimen is within 10 hours of collection.	Complete Blood Count (CBC) with Differential
Platelet Count less than 100,000/µL Updated November 2021	Immature platelet fraction (IPF)
Sickle Cell Screen	Hemoglobin Electrophoresis
Hematology – Optional	
Initial Test and Result	**Optional Follow up Testing**
CBC order → CBC specimens that fulfill criteria listed in Pathologist Review	Pathologist review
CBC w/ Diff → CBC specimens that fulfill criteria listed in Pathologist Review	Pathologist Review
CBC w/ Diff → WBC less than 3.0 or greater than 18.0 HGB less than 8.0 or greater than 18.0 MCV less than 75.0 or greater than 110.0 (updated 04/2021) Absolute neut count less than 1.50 or greater than 9.00 Absolute lymph count less than 0.39 or greater than 4.50 Absolute mono count greater than 1.50 Absolute eos count greater than 1.00 Absolute bas count greater than 0.20 Abnormal instrument flags suggesting abnormality	Manual WBC differential
Cell Ct only BFL order → Body fluid specimens that fulfill criteria listed in Pathologist Review	Pathologist review

Microbiology Reflex Testing

Microbiology – Mandatory	
Initial Test and Result	**Confirmation Testing/Additional Workup**
Anaerobic Culture	Aerobic culture on all orders that do not already have an aerobic culture ordered on the same specimen.
Blood Culture; if positive for growth of bacteria or yeast	• Organism identification will be performed if growth occurs any bottle. • Antimicrobial susceptibility testing will be performed depending on organism identification as per protocol.
Body Fluid culture greater than 1mL sample with only aerobic culture	Add anaerobic culture
Culture from catheter tip or foreign bodies	Foreign body culture
Positive culture for pathogen or organism with clinically significant concentration (bacteria or yeast)	Susceptibility and typing as necessary.
Positive Group B Strep, Penicillin allergy, PCR	Susceptibility testing.
Tissue Specimens ordered as a Body Fluid Culture	Cancel and Order as a Tissue Culture
Body Fluid Specimens ordered as a Tissue Culture	Cancel and Order as a Body Fluid Culture
Mycobacterium tuberculosis Complex PCR	AFB Culture with Smear

Toxicology Reflex Testing

Toxicology – Mandatory	
Initial Test and Result	**Confirmation Testing/Additional Workup**
For obstetric inpatients (Mothers and their babies): Positive Amphetamine, Cannabinoids, Ethanol, Methadone, opiates, Oxycodone or cocaine on a Drug of Abuse screen. For OB 330 Residency: any positive analytes on a Drug of Abuse screen.	LC/MS Confirmation

Transfusion Medicine/Blood Bank Reflex Testing

Transfusion Medicine – Mandatory	
Initial Test and Result	**Confirmation Testing/Additional Workup**
Antibody Screens	ABO/RH
Antibody Titer	ABO/Rh and Antibody Screen
Positive Antibody Screen, or a positive Direct Antiglobulin Test (DAT) on inpatients, outpatients, and surgical patients	Sent to Versiti if antibody and/or antigen testing is needed.
Positive prenatal Profile Type & Antibody Screen	Antibody identification with titer if identified antibody is clinically significant
Women of childbearing age identified as RHD variants or "weak D phenotypes" via serological testing with no previous RHD genotyping on file.	Sent to Versiti for D Variant testing if indicated
Type & Screen (T&S) on a patient with autologous or directed units	Crossmatch of the units and sent to Versiti for testing if indicated
Type & Screen (T&S) on a pre-op patient with an antibody	Crossmatch of two antigen negative units
Patients with difficult antibody situation (e.g., red cell autoantibodies, multiple red cell antibodies or atypical serologic difficulties due to medication, rare antisera or broad serologic reactivity)	Sent to Versiti for further testing
Cord Blood Evaulation/newborn evaluation with negative Rh	Perform Weak D Testing

Urinalysis Reflex Testing

Urinalysis – Mandatory	
Initial Test and Result	**Confirmation Testing/Additional Workup**
UA or UA culture if with inadequate volume for microscopic exam	Urine Dipstick (U dip)
Urinalysis – Optional	
Initial Test and Result	**Optional Follow Up Testing**
If urinalysis (UA) with two or more of the following abnormal findings, provided there are less than 10 squamous epithelial cells observed per high power field: -Greater than or equal to 10 WBC -Positive leukocyte esterase -Positive nitrite OR if specimen is -Grossly bloody	Urine Culture
If volume is inadequate for microscopic exam and Urine Dipstick (U dip) with one or more of the following abnormal findings: -Positive leukocyte esterase -Positive nitrite	Urine Culture