



Corewell Health East Laboratory

Dearborn – Farmington Hills – Grosse Pointe – Royal Oak – Taylor – Trenton – Troy – Wayne

ALGORITHMS FOR REFLEX TESTS

CLINICAL PATHOLOGY

ABO Grouping:

- If patient appears to be group A or AB with unexpectedly positive reverse typing, then ...
 - Reflex testing: special antigen typing for A1 antigen, and antibody identification
 - Process followed for group AB patients also.
- If patient appears to be group B with unexpectedly positive reverse typing, then...
 - Reflex testing: antibody identification.

Acid Elution: (Royal Oak)

- The laboratory performs the test on trauma cases and may reflex to flow cytometry for confirmation by the fetal RBC assay.

Amniotic fluid AFP:

- If elevated AFP MoM or there is a specific clinical history, sample sent to an outside lab for repeat AFP and if necessary, acetylcholinesterase testing.

Anaerobic culture:

- If only an anaerobic culture is ordered a matching aerobic culture will be added.

Anti-Nuclear Antibody (ANA) evaluation:

- Run ANA screen by immunofluorescence
- If greater than cut-off, immunofluorescence microscopy performed - pattern and titer reported.

Anti-Nuclear Antibody (ANA) evaluation with reflex:

- Run ANA screen by immunofluorescence
- If result is positive, report titer and pattern AND reflex to specific autoantibodies for SSA, SSB, Smith, RNP and dsDNA.

Antibody Identification:

- If a new antibody specificity is identified, then...
 - Reflex testing: Special Antigen Typing of patient's RBCs for each specificity (may result in multiple charges).
- If autologous control is positive during antibody identification, then...
 - Reflex testing: Direct Antiglobulin Test x1 (DAT X3 polyspecific, monospecific IgG and C3d done if poly is positive).
- If prenatal patient has or has had clinically significant antibody identified, then...
 - Reflex testing: Antibody Titer q 3-4 weeks. If multiple clinically significant antibodies are present, then a titer is performed for each specificity resulting in multiple charges.
- If antibody activity includes a broadly reactive cold or warm antibody interfering with determination and identification of alloantibodies or crossmatches, then...
 - Reflex testing may include: Adsorptions, Pretreat RBC w/ Enzymes and/or Pretreat Serum by Dilution and/or Pretreat Serum by Diff, Red Cell Adsorptions and Antibody Identification, antigen typing of the patient. Multiple charges may be generated for each sample depending upon complexity of the serological problem.
- If an Antibody Identification is ordered and the patient has no previous history of antibody, then...
 - Indirect Coombs (Antibody Screen) is performed first.

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ALGORITHMS FOR REFLEX TESTS

3 of 9

- If an Antibody Titer is ordered, and the patient has not had antibody identification performed within the last month, then...
 - Antibody Identification is performed first.

Antibody Screening (Indirect Coombs):

- If patient has no previous history of a positive antibody screen, then...
 - Reflex testing: Antibody Identification. Multiple antibody identification panels may be performed in order to elicit antibody specificity.
- If patient has previous history of a positive antibody screen, and ...
 1. Time elapsed since the last antibody identification is
 - greater than 1 month for an obstetrical patient, or
 - greater than 3 months for a non-obstetrical patient, OR
 2. Reactivity occurs with RBCs known to lack the corresponding antigen, OR
 3. Reactivity has increased in strength since the last antibody screen, OR
 4. Patient has a previous history of a WAA (warm auto-antibody) that is currently reactive in the antibody screen OR
 5. Patient is incompatible when crossmatched with antigen negative RBCs, then...
 - Reflex testing: Antibody Identification. Multiple antibody panels may be performed in order to elicit antibody specificity(ies)
- If patient has or previously has had a positive antibody screen (not due to Rh Immune Globulin administration) then...
 - Reflex testing: two (2) RBCs are crossmatched in addition to crossmatch orders.
- If obstetrical patient has history of or current clinically significant antibody, then...
 - Reflex testing: cord blood survey consisting of Direct Antiglobulin Test, Typing ABO, Typing RHO (D), Special Antigen Typing. Multiple charges for Special Antigen Typing if mother has multiple clinically significant antibodies.

Blood Culture:

- Rapid blood culture identification with the BCID2 will be performed on the first positive blood culture per admission. Subsequent positive blood cultures will not be tested with BCID2 unless a new organism or morphology is seen on the Gram stain.

Bone Marrow:

- Ancillary testing, including special stains, immunoperoxidase stains, flow cytometry, cytogenetics, FISH and molecular testing will be performed for diagnostic purposes, on bone marrows and other hematopoietic specimens, as determined necessary by the pathologist to render a diagnosis.
- Cytogenetics, FISH and molecular testing will be performed for prognostic and treatment purposes, based on DMT guidelines (Appendix I).

Clostridium difficile CDH/Toxin NAA testing:

- In cases where GDH is negative and Toxin A/B is positive, NAA testing is performed.

Complete Blood Count (CBC):

- If results are abnormal and meet criteria for requiring pathologist review, add differential. The slides, instrument printout and lab printout are saved for pathologist review
 - Exception: It is not required to add a differential if sending for MCV < 59.5 fL, MCV ≥ 115.0 fL, or PLT >1,000 10⁹/L.

Complete Blood Count with Differential (CBC/diff):

- If results are abnormal and meet criteria for requiring pathologist review, the slides, instrument printout, and lab print out are saved for pathologist review.
- If sickle cells are found on a differential, a sickle cell screen is performed if no previous history.
- If suspect microorganisms are seen on smear, refer to Microbiology for confirmation.

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Crossmatching:

- If patient has clinically significant antibody(ies), then...
 - Reflex testing: Unit Antigen Screen with antisera. Multiple charges generated (# of units tested x number of antigens tested for)...
- If patient with a previous history of clinically significant antibody has incompatible crossmatches with units known to lack corresponding antigen(s), then...
 - Reflex testing: Antibody Identification
- If patient has incompatible crossmatches due to broadly reactive warm or cold auto or allo antibody, then...
 - Reflex testing: Unit Antigen Screen with patient sera, unit antigen screen with reagent anti-sera, phenotypically similar matched units or least incompatible. Multiple charges will be generated equal to number of units tested to locate compatible or least incompatible.
- If patient has a history of Sickle Cell Anemia/Thalassemia then...
 - Units will be confirmed Hemoglobin S negative (additional charge per unit), and antigen negative (C, E, Kell) per patient antigen typing. Multiple charges generated (# of units tested x number of antigens tested for)

Cryoglobulins:

- If a positive cryoglobulin is detected, cryoglobulin characterization and quantitation will be performed automatically the first time the cryoglobulin is detected. Characterization will not be repeated for a given patient unless it is clinically appropriate.

Cryptococcal Antigen Test (CSF only):

- Reflex to include a culture (if not ordered) - College of American Pathologists guideline

CSF cell count and differential:

- All CSF specimens with WBC counts within the normal range (≤ 5 WBC/mcL) will have a CSF scan differential performed. RBCs, PMNs and mononuclear cells will be reported if present.
- If the CSF WBC count is above the normal range (> 5 WBC/mcL), a differential will be performed.
- If results are abnormal and meet criteria for requiring pathologist review, the slides and lab print out are saved for pathologist review.
- If suspect microorganisms are seen on the smear, refer to Microbiology for confirmation.

Direct Antiglobulin Test:

- If Direct Antiglobulin Test is positive for IgG, and patient is baby of mother with clinically significant antibody, then...
 - Reflex testing: Antibody Identification on eluate from baby's RBCs.
- If Direct Antiglobulin Test is positive on post transfusion reaction specimen, then...
 - Reflex testing: Antibody Identification on eluate from patient's RBCs.
- If a Direct Antiglobulin Test is ordered on a patient > 4 months of age, then...
 - DAT is performed using polyspecific AHG. If positive then reflex test: 2 additional DATs IgG and C3 (monospecific).
- If Direct Antiglobulin Test is positive within two weeks of transfusion, then...
 - Reflex Testing: Transfusion Reaction Evaluation if deemed necessary by pathologist

DRVVT (screen and confirm):

- A dRVVT screen is performed. If screen is abnormal, a confirmation test is performed on the patient and a control. Normalized ratio of screening test to confirmation test is calculated and reported.

Fetal Cell Screening:

- If post-partum mother is Rh(D) negative and baby is Rh(D) positive, then...
 - Reflex testing: Fetal Cell Screen

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- If the fetal cell screen is positive, mother or baby is weak D positive, OR baby's Rh type cannot be determined (e.g. no specimen, invalid Rh testing results), then...
 - Reflex testing: Fetal RBC Assay (FMH) ordered by Blood Bank.

Fluid cell count and differential:

- Body fluid differentials will be performed when the WBC count is >5 mL.
- If results are abnormal and meet criteria for requiring pathologist review, the slides and lab print out are saved for pathologist review.
- If suspect microorganisms are seen on the smear, refer to Microbiology for confirmation.

Group A Strep Screen:

- If positive, no additional testing is performed unless specifically requested
- If negative, reflex to Group A Strep by Nucleic Acid Amplification for ages < 16 years.

Group B Strep Screen (Pen Allergy) (*Streptococcus agalactiae*):

- A vaginal/rectal screen reflex to include susceptibility testing.

HBsAg:

- Confirmed when result meets criteria of >1.00 - <50.00. If >50, then not required.

HCV antibody testing for inpatient "source" cases:

- All positives are tested for HCV RNA Quantitation by PCR

Hepatitis C Screening (Effective: 3/1/2022)

- Hepatitis C virus (HCV) quantitative nucleic acid amplification test will be automatically added to all positive/reactive hepatitis C virus antibody tests.

Hemoglobinopathy evaluation:

- Run capillary electrophoresis
- If abnormal hemoglobin detected, run alkaline and acid gel electrophoresis

Hexagonal Phase Phospholipid (screen and confirm):

- A hexagonal screen is performed. If the screen is abnormal, a confirmation test is performed on the patient. The difference between the screen and confirmation is calculated and reported.

HIV antigen/antibody testing:

- HIV 1/2 antibody and p24 HIV-1 antigen test used as initial screen. If positive, additional testing performed using BioRad Geenius.
- If Geenius is negative or equivocal, sample(s) sent to outside lab for HIV-1 RNA.

HLA: HLA antibody identification ordered and performed when the HLA (cytotoxic) antibody screen is positive.

HLA B27:

- If flow result is in equivocal range, refer to HLA Class 1 Disease Association.

IgG subclasses: Total IgG included

Immature Platelet Fraction (IPF):

- Reflex when platelet count is less than 100,000, once every 24 hours.

Inhibitor coagulation screen:

- PT and PTT prolonged. If the PTT is prolonged, a thrombin time is performed to rule out drug effect. Heparin absorption may be performed as needed. If PT is prolonged or the PTT is prolonged without evidence of heparin, then 1:1 mixing studies are performed both immediately and after 120 min incubation at 37C. Additional testing and/or pathologist interpretation is performed as needed.

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6 of 9

Lactic Acid

- When an initial plasma lactic acid has a result > 2.0 mmol/L (i.e. 2.1 or greater), a STAT redraw order for a 2nd plasma lactic acid will occur automatically. This will be scheduled for 2 hours after the initial draw. If the 2 hour is >2.0 mmol/L it will reflex to a 4 hour. The reflex will apply to emergency center and in-patients; it will not apply to any outpatient requests.

Lupus Anticoagulant:

- If this is ordered it reflexes to “Coagulation consult for anti-phospholipid antibody.”

Lyme Antibody:

- All positive or equivocal results will be sent out for Immunoblot testing.

Meningitis/Encephalitis Panel:

- Add cryptococcal antigen if ME Panel is positive for cryptococcus

Molecular Genotyping:

- Molecular Genotyping (send out test) will be performed (additional charge generated):
 - When a patient has 3 or more Alloantibodies OR
 - Patient has Sick Cell/Thalassemia OR
 - Patient is a Warm Autoantibody patient OR
 - Patient being treated for Multiple Myeloma (Daratumumab)

Molecular RhD Genotyping:

- Will reflex on pregnant patients identified as a potential RHD variants or “weak D phenotypes” via serological testing when no previous RhD genotyping is on file.

Peripheral Smears with Microorganisms:

- Reflexed to Microbiology for further evaluation.

Protein C:

- Protein C activity is performed. If abnormal and the patient is not on Coumadin, it is verified with a protein C antigen.

Protein S:

- Protein S activity is performed. If abnormal and the patient is not on Coumadin, it is verified with a protein S free and total antigen.

Serum monoclonal gammopathy evaluation:

- Run SPE and FLC
- Run IFIX if clinician marks indication as amyloid/neuropathy or POEMS. Pathologist may also add IFIX if needed.

Serum protein electrophoresis: all patients

- Run IFIX if clinician marks indication as amyloid/neuropathy or POEMS. Pathologist may also add IFIX if needed.

Sickle Cell:

- If sickle cell screen is positive, Hemoglobinopathy evaluation is reflexed.
- A hemoglobinopathy evaluation is reflexed if a variant is present on hemoglobin quantitation and the patient does not have a previous hemoglobin evaluation in the system.

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Syphilis Screening (Reverse Algorithm)

- Initial screening for total antibody (IgG and IgM) specific for *Treponema Pallidum* using a chemiluminescent immunoassay is performed.
- If total antibody is reactive, an RPR will be performed.
- If total antibody and RPR results are discordant (i.e. total antibody reactive, RPR nonreactive), a TP-PA test will be performed.

Smear Blood Parasite:

- Includes Malaria antigen screen when indicated

TSH with reflex to free T4:

- TSH performed
- If TSH falls outside specified limits, a free T4 is automatically performed.

Urinalysis:

- Dipstick testing is performed; if positive result encountered (other than urobilinogen), automated and/or manual microscopy is performed.

Urine protein electrophoresis:

- Run UPE and kappa/lambda immunofixation
- Run complete immunofixation at Pathologist's discretion or if new abnormality

Yeast – Antimicrobial sensitivity testing (AST) will be performed on all yeast recovered from sterile sites (including blood cultures).

ANATOMIC PATHOLOGY

In the Anatomic Pathology laboratory, pathologists use their judgement to order and examine additional studies on submitted specimens if they deem them medically necessary to render a diagnosis. In these situations, pathologists are acting in the capacity of a consultant in the care of the patient. This includes but is not limited to ordering special stains, decalcification of tissue, immunoperoxidase stains, microbiology cultures on tissue specimens, electron microscopy, and flow cytometry on certain tumors if indicated.

The Anatomic Pathology reflex test list includes tests that Beaumont Laboratory performs if a specimen meets the reflex criteria listed and tests that are often useful for the diagnosis and prognosis of the patient. Treating providers may decline the reflex testing by indicating that on the laboratory requisition or by contacting Beaumont Laboratory's Customer Service Department.

ALK

- Performed on all lung resections (wedge resection, segmentectomy, lobectomy and pneumonectomy) with non-small cell lung carcinoma (NSCLC). Primary testing ALK (D5F3) immunohistochemistry (IHC). Reflex to ALK FISH if needed.

BCR/ABL1 for t(9;22)(q34;q11.2) detection

- When a BCR/ABL1 Dual Color Dual Fusion Translocation probe produces an atypical signal pattern (1R1G1F in >23% of nuclei) indicating the arginosuccinate synthetase (ASS) gene on chromosome 9q34 may be deleted, reflex testing to the BCR/ABL1 + 9q34 Tri-Color Dual Fusion probe should be performed to determine if there is an ASS gene deletion present.

Estrogen and Progesterone Receptor Status (IHC)

- Performed on all newly diagnosed invasive and recurrent/metastatic breast cancers to assess responsiveness to endocrine therapy

Epidermal Growth Factor Receptor EGFR (PCR) Mutation Analysis (Effective: 3/1/2022)

- Performed on all lung resections (wedge resection, segmentectomy, lobectomy and pneumonectomy) positive for non-small cell lung carcinoma (NSCLC) tumors

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Fluorescence *in situ* hybridization (FISH) Panels

- All FISH panels are performed according to the DMT guidelines (Appendix I).

Her2-Status (IHC and FISH)

- Performed on all newly diagnosed and recurrent invasive breast cancers, and metastatic tumors, to guide the decision to pursue Her2-targeted therapy.
- Performed on all newly diagnosed gastric, gastroesophageal junction adenocarcinomas and their recurrences/metastases
- Performed on all newly diagnosed distant metastases of colorectal carcinoma.

IDH1 (Isocitrate Dehydrogenase) (IHC)

- Performed on all infiltrating gliomas for diagnostic and prognostic purpose

MGMT (O6-Methylguanine-DNA Methyltransferase (**MGMT**)) Methylation Status

- Biomarker in pediatric and adult patients with glioblastoma, for diagnostic and therapy guiding purpose

Mismatch Repair Analysis (IHC)

- Performed on all newly resected colorectal carcinomas and endometrial carcinoma as a screen for Lynch syndrome
 - MLH1 Promotor methylation is ordered on specimens that show a loss of MLH1 protein expression on immunohistochemistry
 - BRAFV600E is reflexively tested on colorectal cancers displaying loss of MLH1 protein expression on IHC and absence of MLH1 promotor methylation
- Performed on any metastatic carcinoma (from any primary organ site) for possible immunotherapy.
- Performed on all new primary site biopsy diagnoses of rectal or rectosigmoid adenocarcinoma.

Aggressive B-Cell Lymphoma Panel

- Performed according to aggressive B-cell lymphoma FISH diagnostic algorithm (DMT guidelines).

PDL-1 (Clone 22C3)

- Performed on all newly diagnosed metastatic non-small cell lung carcinoma (NSCLC subtypes: adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, large cell neuroendocrine carcinoma, NSCLC favor squamous cell/adenocarcinoma, or NSCLC-NOS).
- Performed on all metastatic carcinoma in which the possibility of a NSCLC cannot be entirely excluded.
- Performed on all lung resections (wedge resection, segmentectomy, lobectomy and pneumonectomy) with non-small cell lung carcinoma (NSCLC).

p16

- Immunohistochemistry (IHC) testing performed on all newly diagnosed head and neck squamous carcinomas arising in the oropharynx (Base of tongue and tonsils)
- IHC testing performed on squamous carcinoma presenting as a lymph node metastasis of unknown primary site

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Appendix I –

GUIDELINES FOR CYTOGENETIC/MOLECULAR FOLLOW-UP TESTING OF HEMATOPOIETIC MALIGNANCY

This summarizes the current testing protocol and ancillary molecular and cytogenetic tests that will automatically be ordered by the pathologists in all follow up specimens of hematopoietic malignancy.

Acute Lymphoblastic Leukemia	• Karyotype • FISH for previously identified abnormality • RT-PCR for BCR-ABL1 if positive at diagnosis
Acute Myeloid Leukemia, including evolving AML	• Karyotype • Repeat mutations (NPM1, FLT3, IDH1/IDH2), on follow up marrows other than day 14. Note: sensitivity may not be sufficient for evaluation of minimal residual disease • PCR for RUNX1-RUNX1T1, PML-RARA etc performed by clinician on peripheral blood
Myelodysplastic Syndrome	• Karyotype • Previously identified FISH abnormality.
Myeloproliferative Neoplasms	• Karyotype
Myelodysplastic/Myeloproliferative Neoplasms including Chronic Myelomonocytic Leukemia	• Karyotype
Plasma Cell Myeloma	• Karyotype • FISH for previously identified abnormality
Chronic Lymphocytic Leukemia/ Small Lymphocytic Lymphoma	• Karyotype • Complete CLL FISH panel
T-cell leukemia, including T-cell Prolymphocytic Leukemia	• Karyotype • FISH for previously identified abnormality
Lymphomas	• Cytogenetic and molecular testing for previously identified abnormality in DLBCL and mantle cell lymphoma • Other testing at physician request

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Appendix II - GUIDELINES FOR CYTOGENETIC/MOLECULAR TESTING OF HEMATOPOIETIC MALIGNANCY

This Laboratory Bulletin summarizes the current testing protocol and ancillary tests that will automatically be ordered by the pathologists in all new diagnoses of hematopoietic malignancy.

Laboratory Bulletin

B Lymphoblastic Leukemia/Lymphoma	- Karyotype - ALL FISH Panel [4,10 trisomy, t(9;22), 11q23 rearrangement, t(12;21), t(1;19), IGH/IL3 for t(5;14), iAMP21, ABL1, ABL2, PDGFRB per Children's Oncology Group protocol] - RT-PCR for BCR-ABL1 - Send out for Phil-like ALL FISH Panel testing (MAYO), at ordering physician request only
Acute Myeloid Leukemia, including evolving AML	- Karyotype - Limited AML FISH Panel [CBFB gene rearrangement for inv16/t(16;16), p53, t(15;17), MLL, t(9;22), NUP98] on all - Expanded FISH panel may also be performed at discretion of pathologist and includes t(8;21) and 3q26 rearrangement - Next Generation Sequencing - Myeloid Panel - FLT3
Myelodysplastic Syndrome and suspicious for myelodysplastic syndrome	- Karyotype - Next Generation Sequencing - Myeloid Panel - FISH only upon request or at discretion of pathologist or if traditional karyotype obtained <20 metaphases [MDS FISH Panel: trisomy 8,del(20q),monosomy 5/del(5q),monosomy 7/del(7q),3q26 rearrangement, monosomy 13/del(13q), MLL,p53]
Myeloproliferative Neoplasms	- Karyotype - FISH or RT-PCR for BCR-ABL1 - JAK2 V617F, CALR, and MPL. Also, JAK2 Exon 12 if concerned for PV - If triple negative (JAK2 V617F, CALR, MPL) a next generation myeloid panel will be performed
Myelodysplastic/Myeloproliferative Neoplasms including Chronic Myelomonocytic Leukemia	- Karyotype - FISH or RT-PCR for BCR-ABL1 - JAK2 V617F, CALR and MPL - Eosinophilia FISH Panel [FIP1L1/PDGFR, PDGFRB, FGFR1 and JAK2 rearrangements] - MDS FISH only upon request or at discretion of pathologist or if traditional karyotype obtained <20 metaphases [MDS FISH Panel: trisomy 8,del(20q),monosomy 5/del(5q),monosomy 7/del(7q),3q26 rearrangement, monosomy 13/del(13q), MLL,p53] - Next Generation Sequencing - Myeloid Panel
Eosinophilia associated with myeloid/lymphoid neoplasms	- Karyotype - Eosinophilia FISH Panel [FIP1L1/PDGFR, PDGFRB, FGFR1 and JAK2 rearrangements]
Plasma Cell Myeloma	Karyotype

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	Myeloma FISH Panel [CDKN2C/CKS1B for chromosome 1q rearrangement, CEP3/7 for hyperdiploidy, monosomy 13/del(13q), p53, IGH/CCND1 with reflex to IGH/FGFR3, IGH/MAF, and IGH/MAFB]
Chronic Lymphocytic Leukemia/ Small Lymphocytic Lymphoma	- CLL FISH Panel [MYB/CEP6, trisomy 12, p53, ATM, monosomy 13/del(13q), IGH/CCND1] - Karyotype
Diffuse Large B-cell Lymphoma	- FISH for double hit lymphoma all cases [MYC and IGH/MYC; Reflex to BCL2 and BCL6 if MYC rearranged] - Karyotype optional
Burkitt Lymphoma	FISH for IGH/MYC with reflex to IGK/MYC and IGL/MYC
Hairy Cell Leukemia	BRAF V600E (send out)
Lymphoplasmacytic Lymphoma	- MYD88 L265P - CXCR4 at request of ordering physician for patient's being considered for ibrutinib
Gastric Malt Lymphoma	H. pylori testing (also should be performed in gastric DLBCL) - If positive for H. pylori; FISH for t(11;18) will be performed
Mantle Cell Lymphoma	P53 by NGS
T-cell leukemia/lymphoma including T-cell Prolymphocytic Leukemia	- Karyotype - T-cell leukemia/lymphoma FISH Panel [TCL1 (14q32), TCRAD (14q11), TCRB (7q434), and TLX3 (5q32) rearrangements; chromosome 8 abnormalities; del(9p)] - T-cell gene rearrangement studies may be performed at discretion of pathologist
Peripheral T-cell Lymphoma and Primary Cutaneous CD30 Positive T-cell Lymphoproliferative Disorders	- FISH for DUSP22 if ALK negative ALCL consideration (paraffin only) - T-cell gene rearrangement studies may be performed at discretion of pathologist
Other lymphomas	- Additional cytogenetic and molecular testing, including B- and T-cell gene rearrangement studies, and FISH for ALK gene rearrangement is useful under certain circumstances and will be performed at discretion of pathologist - NHL FISH Panel (IGH/CCND1, IGH/BCL2, MALT1, MYC, BCL6)

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