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Essential Thrombocythemia

Essential thrombocythemia (ET) is a somewhat rare (<3/100,000/year) chronic myeloproliferative disorder (CMD). Like polycythemia vera (PV), another rare CMD (see April 2003 *Communiqué*), ET causes increased cell production. However, while PV causes an increase in the red cell component and can sometimes increase the platelet and white cell count, ET causes an increase in only the number of platelets. This increase in platelet count results in clotting (thrombosis), as well as bleeding problems that result from abnormal platelet function.

As with other CMDs, ET usually affects older adults between the ages of 50-70, although it can occur in younger patients. ET usually is an indolent disorder, with patients' lifestyles unaffected for many years. Conversely, some patients present with severe thrombohemorrhagic complications that require immediate intervention. However, even if the disease is asymptomatic, monitoring, and possibly treatment, should be performed to minimize risks of thrombosis. Additionally, as with other CMDs, in rare cases (<5%) ET will progress to acute leukemia and patients should be monitored for signs of this progression.

Unlike the other CMDs, ET cannot be diagnosed by any particular tests. It must be diagnosed as a result of exclusion of those factors that would identify the disorder as one of the other myeloproliferative disorders.² A complete medical history is necessary to appropriately evaluate the patient.²

Diagnosis of ET

Because of the low frequency of symptoms in early disease, most cases of ET will be identified as the result of incidental findings on a routine examination. Patient history combined with the complete blood count (CBC) and peripheral smear provide the foundation for the diagnosis. An elevated platelet count should be confirmed with a second blood test and a manual peripheral blood smear examination.

When a patient presents with thrombocytosis, the physician must first rule out a reactive process. Reactive thrombocytosis may occur as a result of surgery, acute pancreatitis, myocardial infarction, burns, infection, acute hemolysis, acute bleeding, coronary artery bypass grafting, or as a rebound from chemotherapy or immune thrombocytopenia.¹ In addition, several chronic conditions may cause reactive thrombocytosis, including:¹

- Iron-deficiency anemia
 - Hyposplenism (either surgical or functional) seen in patients with sickle cell anemia, hereditary spherocytosis, some hemoglobinopathies, amyloidosis, and some autoimmune diseases
 - Metastatic cancer or lymphoma
 - Inflammation due to rheumatoid arthritis, vasculitis, or allergies
 - Renal failure or nephrotic syndrome
- Additionally, one must always consider that patients with reactive thrombocytosis also may have ET.

Once reactive thrombocytosis has been eliminated from the diagnosis, the physician must rule out the other myeloproliferative disorders including polycythemia vera, chronic myelocytic leukemia, agnogenic myeloid metaplasia, acute leukemia, and myelodysplasia.² The usual symptoms of ET are associated with circulatory problems: clotting, bleeding, abnormal small-blood-vessel circulation, and splenomegaly. Patients may have any of the following symptoms:

- Pain, tingling, and burning sensations in the extremities (acroparesthesias)
- Redness or swelling in extremities (erythromelalgia)
- Chest pain
- Coughing
- Bleeding
 - Easy bruising
 - Bleeding of gums and mucous membranes
 - Blood in stools or urine
 - Bleeding for no apparent reason
 - Petechiae on the extremities

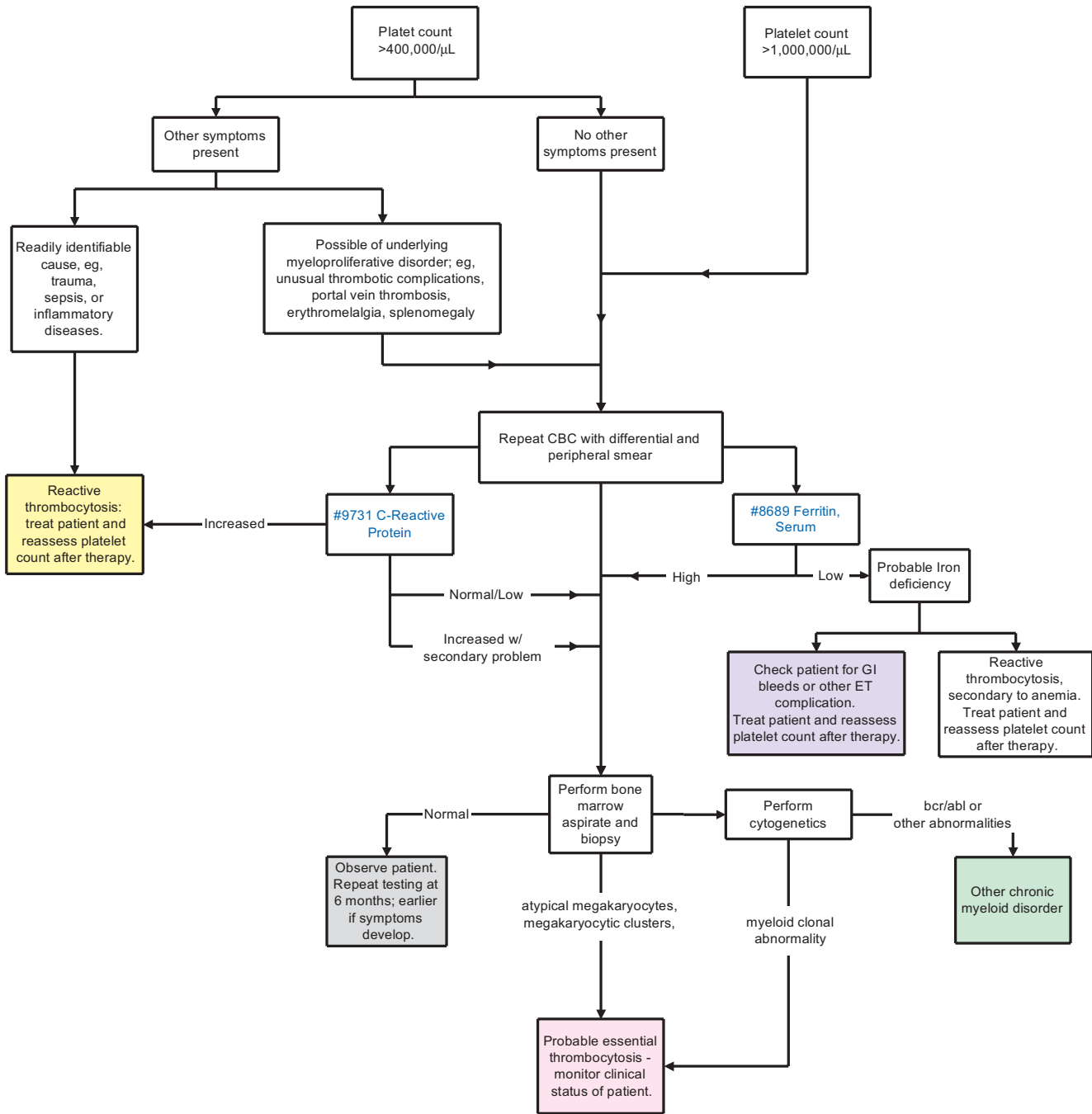


Figure 1. Laboratory Diagnosis of Essential Thrombocytosis

- Signs of stroke or transient ischemic attack (TIA):
 - Headache
 - Dizziness
 - Changes in vision
 - Confusion in speech
 - Numbness in extremities

Clinical features of ET also may include Budd-Chiari syndrome (thrombosis of the hepatic vein with great enlargement of the liver and extensive development of collateral vessels, intractable ascites, and severe portal hypertension) and any history of deep venous thrombosis.

An algorithm is provided that diagrams the tests required to reach a diagnosis of ET. See Figure 1.

A serum ferritin level, [#8689 Ferritin, Serum](#), should be determined to exclude the possibility of iron deficiency as the cause of thrombocytosis.²

C-reactive protein levels (CRP), [#9731 C-Reactive Protein, Serum](#), should be examined. CRP levels usually are normal in uncomplicated ET and may be elevated in reactive thrombocytosis.¹ However, elevated CRP levels may be seen in patients with ET with secondary problems.

Additionally, a bone marrow examination with an aspirate, biopsy, and cytogenetic studies should be performed to aid in the diagnosis of ET. Cytogenetic studies for ET most commonly involve performing [#80578 Fluorescence In Situ Hybridization DNA Probes \(D-FISH\) with BCR/ABL](#) to rule out chronic myelocytic leukemia. ET also has been associated with +8 and +9 chromosome abnormalities (detected by [#8506 Chromosome Analysis, For Hematologic Disorders, Bone Marrow](#)). Bone marrow analysis should demonstrate increased and atypical megakaryocytes and megakaryocytic clusters in ET.¹

Treatment

Treatment for ET may frequently be described as watchful waiting. However, depending upon the patient's risk of thrombosis, treatment may include platelet apheresis, chemotherapy, or other medications. For the patient who presents with very high platelet counts (>1 million/ μ L) and who is at high risk of an adverse event (patients who have a history of bleeding or clotting complications), an emergency hematology consultation is advised. In addition, these patients should be considered for platelet apheresis.²

As a rule, asymptomatic younger patients with low risk of vascular complications may not require treatment. However, for older patients (>60 years), the treatment goal is to lower the platelet count to <400,000 μ L (platelet reference range = 150,000 to 450,000). When therapy for ET is deemed necessary, platelet reduction can be accomplished by myelosuppressive treatment. The most common form of chemotherapy involves oral hydroxyurea. This drug lowers the platelet count by suppressing the bone marrow's platelet production. It also lowers red and white cell counts. Hydroxyurea has been shown to decrease the risk of thrombohemorrhagic complications, but it may slightly increase the risk of long-term transformation of ET to acute leukemia.

Anagrelide hydrochloride is another platelet-reducing agent that may be given to control the risk of thrombosis and bleeding. Anagrelide, the only drug approved by the Food and Drug Administration for platelet reduction in CMD, is felt to be safe for the long term, but expense and toxicity limit its use. Anagrelide reduces the platelet count by interfering with megakaryocyte maturation. Interferon-alpha suppresses proliferation of leukocytes and controls thrombocytosis, splenomegaly, and disease-associated symptoms¹ and also may be of benefit in certain cases. Toxicity with this treatment may be reduced by the recently released pegylated formulation.

Platelet apheresis treatment, where the blood is removed, the platelets are filtered out, and the blood is returned to the body, rapidly reduces the platelet count, decreasing the risk of thrombosis and bleeding. This procedure is only performed in the face of emergent or life-threatening complications.

Low-dose (81 mg/day) aspirin also may aid in alleviating the symptoms of ET. In many cases, aspirin may be all that is required to control the headache, erythromelalgia, acroparesthesia, and visual symptoms experienced by some patients.¹

Conclusion

While a diagnosis of ET is not necessarily life changing for the patient, the risks of thrombosis, bleeding, and progression to acute leukemia require that the physician closely monitor these patients. When symptoms do present, treatment options are relatively straightforward and effective.

References

1. Tefferi A, Silverstein MN: Myeloproliferative Diseases. In Cecil Textbook of Medicine, 21st edition. Edited by L Goldman. WB Saunders Co, 2000, pp 935-941
2. Tefferi A: Chronic Myeloproliferative Diseases. In Primary Hematology. Edited by A Tefferi. Totowa, NJ, Humana Press, 2001, p 119-148

CMV/HSV - Additional Specimen Types Validated

Mayo's Virology Laboratory has validated additional sources for cytomegalovirus (CMV) and herpes simplex virus (HSV) by rapid polymerase chain reaction (PCR). The following chart lists all approved specimen types and required quantities.

Preferred transport temperature is refrigerate.

Specimen Types for Rapid PCR Testing										
Test #	Virus	CSF*	Dermal	Genital	Body fluids	Respiratory fluids	Throat	Urine	Plasma	Tissue** Other than dermal
81240	CMV	0.5 mL	-	-	0.5 mL	0.5 mL	swab	2.0 mL	2.0 mL	Yes
80575	HSV	0.5 mL	swab/ biopsy	swab	0.5 mL	0.5 mL	swab	-	-	Yes
81239	EBV	0.5 mL	-	-	-	-	-	-	-	No
81241	VZV	0.5 mL	swab/ biopsy	-	-	-	-	-	-	No
80066	Enterovirus	1.0 mL	-	-	-	-	-	-	-	No
80067	JC/BK	1.0 mL (JC only)	-	-	-	-	-	1.0 mL (BK only)	1.0 mL (BK only)	No

*CSF = cerebrospinal fluid

**Send tissue refrigerated in a screw-capped, sterile container containing 1.0-2.0 mL sterile saline or multi-microbe medium (M5)

Alpha-Globin Gene Analysis Expanded

[#9499 Alpha-Globin Gene Analysis](#) utilizes direct mutation analysis by a Southern blot procedure to detect deletions within the alpha-globin locus that result in alpha-thalassemia. Recently, Mayo added a polymerase chain reaction (PCR)-based assay to detect the Thailand (Thai) and Filipino (Fil) deletions. The addition of the PCR method also results in changes to the CPT codes.

New CPT Codes:

83891/DNA Extract-purify
83892/x3 Enzyme digestion
83894/x7 Electrophoresis
83896/x2 Nucleic acid probe, each
83897/x2 Nucleic acid transfer
83901/Each multiplex PCR
83912/Interpretation and report

Previous CPT Codes:

83891/DNA Extract-purify
83892/x3 Enzyme digestion
83894/x2 Electrophoresis
83896/x2 Nucleic acid probe, each
83897/x2 Nucleic acid transfer
83912/Interpretation and report

Lyme Evaluation Reference Value Change

The reference values for [#82516 Lyme Disease Serology Evaluation, Serum](#) will change to more accurately interpret the result: Nonreactive will change to Negative and Reactive will change to Positive. Positive specimens will continue to automatically reflex to an antibody confirmation by immunofluorescence assay and Western blot.

Hepatitis Be Method and Reference Value Changes

The hepatitis Be antibody and antigen tests have changed from the manual DiaSorin kit to an automated DiaSorin platform. This change improves turnaround times: previously 2-day reporting time, the new method will allow reporting of results in 1 day for the individual tests. In addition, the reference values will expand to include an equivocal result. These changes will affect the following tests:

[#80510 Hepatitis Be Antigen \(HBeAg\), Serum](#)
[#80973 Hepatitis Be Antibody \(Anti-Hbe\), Serum](#)
[#9023 Chronic Hepatitis Profile \(Type B\), Serum](#)
[#5566 Prenatal Hepatitis Profile, Serum](#)
[#8311 Hepatitis BeAg and Antibody, Serum](#)

Cystine Test Changes

The cystine test ([#8376 Cystine, Quantitative, Urine](#)) was developed to aid in the diagnosis of cystinuria. Cystinuria is a disorder of the transport of 4 amino acids: cystine, lysine, arginine, and ornithine, in the intestine and kidney tubules. The test has been changed to [#8376 Cystinuria Profile, Quantitative, 24 hour](#) to include quantitation of the 4 amino acids. An interpretive report will be provided when appropriate. Please note that this test requires a 24-hour urine specimen. With the implementation of these changes, [#8377, Cystine, Qualitative, Random Urine](#), has been deleted.

New Reference Values

CYSTINE

3-15 years: 11-53 $\mu\text{mol}/24$ hours
 ≥ 16 years: 28-115 $\mu\text{mol}/24$ hours

LYSINE

3-15 years: 19-140 $\mu\text{mol}/24$ hours
 ≥ 16 years: 32-290 $\mu\text{mol}/24$ hours

ORNITHINE

3-15 years: 3-16 $\mu\text{mol}/24$ hours
 ≥ 16 years: 5-70 $\mu\text{mol}/24$ hours

ARGININE

3-15 years: 10-25 $\mu\text{mol}/24$ hours
 ≥ 16 years: 13-64 $\mu\text{mol}/24$ hours

Conversion Formulas:

Result in $\mu\text{mol}/24$ hours $\times 0.24$ = result in mg/24 hours
Result in mg/24 hours $\times 4.17$ = result in $\mu\text{mol}/24$ hours

New CPT Code

82136

Previous CPT Code

82127

82131

Metanephrines Method and Name Change

[#81609 Metanephrines, Plasma](#) was converted from a high-performance liquid chromatography (HPLC) method to a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The new method is less prone to interferences and decreases the turnaround time from 4 days to 2 days, improving patient care. The method change does not impact the reference values. In addition to the method change, the name was changed to [#81609 Metanephrines, Fractionated, Free, Plasma](#) to more accurately reflect the analytic performance of the test.



Q: Can Mayo provide a numeric risk calculation for Down syndrome in diabetic pregnancies?

A: An accurate patient-specific risk for Down syndrome in diabetic patients cannot be provided; however, a pseudo-risk can be calculated by dividing the multiple of median (MoM) value for each marker by the corresponding median MoM in unaffected diabetic pregnancies. The adjusted MoM values are then used in the risk calculation. Adjusting the MoMs in this way allows for differences in levels of the markers between diabetic and nondiabetic women. The pseudo-risk is then compared with the risk cutoff to classify the result as screen positive or screen negative. The pseudo-risk is not the woman's true risk of having a pregnancy with Down syndrome. Neither the true risk nor the detection rate can be calculated, because the distributions of the serum markers in diabetic pregnancies with Down syndrome are not known.

Q: How should a positive screen for Down syndrome for women over age 35 be interpreted?

A: The Down syndrome screen risk is derived by combining the maternal age-related risk and analyte levels for the current pregnancy. The 1/270 screen cutoff for Down syndrome applies to all age groups; therefore, if a calculated Down syndrome screen risk is 1/270 or higher it will be classified as screen positive, regardless of whether or not it is less than the age-related risk. The calculated screen risk is more accurate than the age-related risk alone, because it incorporates both the maternal age and marker values.

Q: Which ultrasound measurement should be used to calculate gestation for maternal serum screening?

A: A crown rump length (CRL) measurement between 6-11 weeks has an accuracy of ± 3 to 5 days. For second trimester measurements, biparietal diameter (BPD) is typically accurate to within ± 7 to 10 days. Therefore, if available, CRL typically provides a more accurate gestation. If ultrasound is not done until second trimester, BPD is preferred to femur length or a composite measurement. Pregnancies affected with Down syndrome often have shortened fetal femur length. This discrepancy in femur length decreases gestational dating, thereby resulting in normalized marker values, which decreases the Down syndrome detection rate.

2003 Meeting Calendar

Interactive Satellite Programs . . .

Seronegative Spondyloarthropathies: Review and Recent Advances

September 16, 2003

Presenters: Nisha Manek, MD & Clement Michet, Jr, MD
Moderator: Steven Ytterberg, MD

Pharmacogenetics and Pharmacogenomics of Antidepressants

October 7, 2003

Presenters: John Black, MD
David Mrazek, MD
Elliott Richelson, MD
Moderator: Robert Kisabeth, MD

Pharmacogenomics of Warfarin Therapy

November 11, 2003

Presenter: John Heit, MD
Moderator: Robert Kisabeth, MD

Cardiac Markers

December 2, 2003

Presenter: Allan Jaffe, MD
Moderator: Robert Kisabeth, MD

Upcoming Education Conferences . . .

Practical Surgical Pathology

September 11-13, 2003

Mayo Clinic, Siebens Building
Rochester, Minnesota

Integration Through Community Laboratory

Insourcing: Implementing a Successful Laboratory Program

October 8-10, 2003

Providence Marriott Hotel
Providence, Rhode Island



For a complete listing of all the courses offered throughout the year, contact the Mayo Reference Services
Education Office at 1-800-533-1710 or 507-284-8742.

Abstracts of Interest

Primer on Medical Genomics Part III: Microarray Experiments and Data Analysis

Ayalew Tefferi, MD; Mark E. Bolander, MD; Stephen M. Ansell, MD, PhD; Eric D. Wieben, PhD; and Thomas C. Spelsberg, PhD

Genomics has been defined as the comprehensive study of whole sets of genes, gene products, and their interactions as opposed to the study of single genes or proteins. Microarray technology is one of many novel tools that are allowing global and high-throughput analysis of genes and gene products. In addition to an introduction on underlying principles, the current review focuses on the use of both complementary DNA and oligodeoxynucleotide microarrays in gene expression analysis. Genome-wide experiments generate a massive amount of data points that require systematic methods of analysis to extract biologically useful information. Accordingly, the current educational communication discusses different methods of data analysis, including supervised and unsupervised clustering algorithms. Illustrative clinical examples show clinical applications, including (1) identification of candidate genes or pathological pathways (ie, elucidation of pathogenesis); (2) identification of "new" molecular classes of diseases that may be relevant in disease reclassification, prognostication, and treatment selection (ie, class discovery); and (3) use of expression profiles of known disease classes to predict diagnosis and classification of unknown samples (ie, class prediction). The current review should serve as an introduction to the subject for clinician investigators, physicians and medical scientists in training, practicing clinicians, and other students of medicine.

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The complete article is available online at URL: <http://www.mayo.edu/proceedings/>

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Leukemia/Lymphoma Immunophenotyping Test Modification

Specimens submitted for [#3287 Leukemia/Lymphoma Immunophenotyping by Flow Cytometry](#) are reviewed by a Mayo pathologist to determine the most appropriate and cost-efficient immunophenotypic testing to perform. A triage panel is initially performed to evaluate for B-cell clonality by kappa and lambda light chain expression and for increased numbers of blasts by CD45 expression and side scatter gating. The triage panel also includes antibodies to assess the number of CD3 positive T-cells and CD16 positive/CD3 negative natural killer (NK) cells present and determines if there are increased numbers of T-cells that aberrantly coexpress CD16, an immunophenotypic feature of T-cell granular lymphocytic leukemia. This minimal panel, together with the provided clinical history and morphologic review, is used to determine what, if any, further testing is needed for disease diagnosis or classification:

- If increased numbers of leukemic blasts are identified by immunophenotypic testing, and/or morphologic evaluation suggests that flow cytometry is needed for leukemia diagnosis and classification, an acute leukemia immunophenotyping panel will be performed.
- If excess kappa- or lambda-positive lymphocytes are identified, then a B-lymphocyte immunophenotyping panel is performed.
- If there are increased numbers of T- or NK-cells or if a CD3 positive T-cell population that coexpresses CD16 is identified, a T-lymphocyte immunophenotyping antibody will be performed. In our laboratory, T-cell immunophenotyping studies always include CD2, CD3, CD5, CD7, CD4, and CD8. Additional antibody panels may be used in the evaluation of potential T- and NK-cell lymphoproliferative disorders. These include: antibody panels ([#82962 Granular Lymphocytic Leukemia Flow Panel \[KIR panel\]](#)) to assess for the expression of additional NK-cell associated antigens (useful in establishing a diagnosis of granular lymphocytic leukemia) and assessment of T-cell receptor beta chain variable region family expression (useful in establishing T-cell clonality, see below [[#80028 T-Cell Clonality by Flow Cytometry of TCR V Beta](#)]).

If no abnormalities are detected by the triage panel, no further flow cytometric assessment will be performed unless otherwise indicated by specific features of the clinical presentation or prior laboratory results.

T-Cell Clonality Detection

Establishing T-cell clonality is a critical laboratory element in the diagnosis of T-cell malignancies such as T-cell lymphoma, T-cell acute lymphocytic leukemia (T-ALL), T-cell large granular cell leukemia (T-LGL), and Sézary syndrome. Most available methodologies utilize molecular technologies such as polymerase chain reaction (PCR) and Southern blot to examine the genomic DNA for clonal T-cell receptor (TCR) gene rearrangement; these techniques are time consuming, expensive, and difficult to perform.

Detection of expanded T-cell clones by flow cytometry can be accomplished utilizing antibodies to the V beta subsets of the TCR. This can be particularly helpful in specimens with low lymphocyte counts, or in cases that could potentially have oligoclonal reactive T-cell populations. Additionally, specific cell populations can be examined for unique V beta expression, which may allow for greater understanding of the significance of the detected T-cell clone and better understanding of the disease process.

The technical performance of #3287 **Leukemia/Lymphoma Immunophenotyping by Flow Cytometry** will not change; it will follow the same consultative ordering algorithm that is currently used. An additional panel (**T-Cell Clonality by Flow Cytometry of TCR V Beta**) is being added to the testing algorithm (in combination with routine T-cell phenotyping) to aid in the diagnosis of T-cell malignancies by:

- Rapidly identifying or confirming T-cell clonality in blood
- Guiding the utilization of the more laborious molecular T-cell gene rearrangement techniques

Regardless of which panel is performed, the immunophenotypic results are correlated and interpreted in conjunction with the clinical history and routine morphologic findings. If necessary, cytochemical stains will be performed at no additional charge to further subclassify the disease process. In some instances, additional cytogenetic or molecular genetic studies may be indicated by the immunophenotyping results. In these cases, the ordering physician will be contacted prior to these studies being performed. Reports will include a morphologic description, a summary of the procedure, percent positivity of selected antigens, and an interpretive conclusion based on the correlation of the morphologic features and immunophenotypic results.