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Feature

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A Combined Cytoplasmic Immunoglobulin Staining and FISH Method (cIg FISH) to Detect Prognostic Genetic Anomalies in Multiple Myeloma and Related Plasma Cell Proliferative Disorders

Multiple Myeloma and Related Disorders

Multiple myeloma is one of several types of cancer resulting from the malignant proliferation of plasma cells. As the disease progresses, the malignant plasma cells proliferate within the bone marrow, resulting in compromise of normal marrow cells and thus depletion of circulating red blood cells (anemia), and in more extreme cases depletion of white blood cells (leukopenia) and platelets (thrombocytopenia). The plasma cells secrete cytokines, which in turn overstimulate osteoclasts and lead to characteristic bone destruction without simultaneous bone reformation. This breakdown results in bone pain, pathological fractures, and hypercalcemia. An additional common feature of myeloma is plasma cell overproduction of monoclonal antibody, which is observed in the serum and/or urine. The combination of monoclonal antibody overproduction and the accumulation of increased calcium from bone breakdown often results in renal insufficiency.^{1,2}

Monoclonal gammopathy of undetermined significance (MGUS) is a stable premalignant plasma cell proliferative disorder (PCPD) that is typically detected in asymptomatic patients during routine laboratory testing. Similar to myeloma, these patients produce a monoclonal antibody, but they do not have the cytopenias, renal insufficiency, or bone lesions seen in myeloma.^{1,3} The site of MGUS origin is unknown, as patients typically have a morphologically normal bone marrow. Two major types of MGUS exist: IgM MGUS and non-IgM MGUS (mostly comprised of IgG and

IgA). Patients with non-IgM MGUS progress to multiple myeloma at a rate of 1% per year.

Multiple myeloma and MGUS are 2 of several clinically distinct PCPDs. Some patients have a less active type of myeloma, known as smoldering myeloma, in which they fulfill the minimal myeloma diagnostic criteria, yet are asymptomatic and have no bone lesions. Other patients have indolent myeloma, which is similar to smoldering myeloma except that patients have up to 3 lytic bone lesions without bone pain, intermediate monoclonal protein levels, and have no sign of infection. Another PCPD is plasmacytoma, in which patients present with a solid "mass" of plasma cells, yet

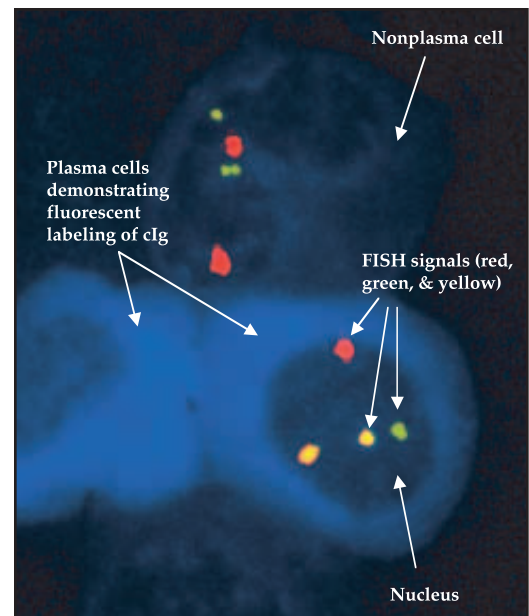


Figure 1. Representative Cells in Cytoplasmic Immunoglobulin (cIg) FISH Studies.

lack significant bone marrow involvement. The plasmacytomas occur primarily in bone or can occur as an extramedullary mass. Additional PCPDs include osteosclerotic myeloma (POEMS syndrome: polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes), plasma cell leukemia, and nonsecretory myeloma. Finally, amyloidosis can occur as a secondary complication of PCPD due to inappropriate deposition of the overproduced monoclonal antibody.²⁴

Testing for Multiple Myeloma

Many clinical and laboratory tests are routinely utilized in the diagnosis, prognostication, and disease tracking of myeloma. These tests include imaging studies, routine diagnostic studies of blood, urine, and bone marrow, as well as genetic testing. Imaging studies, such as X-ray and/or MRI, are used to identify the characteristic bone lesions seen in myeloma. Electrophoresis and immunofixation of blood and urine proteins are utilized to identify the monoclonal antibodies observed in these fluids. Evaluation of blood and bone marrow by hematopathology is also

undertaken to determine the plasma cell burden, cell morphology, and to document various cytopenias. Additionally, the plasma cell labeling index is used to quantify the number of actively dividing plasma cells to distinguish between multiple myeloma and other PCPDs. Prognostic tests include measuring serum levels of beta₂ microglobulin, c-reactive protein, lactate dehydrogenase, and markers associated with the characteristic bone breakdown; research tests such as interleukin-6 and soluble interleukin-6 receptors; as well as testing for specific genetic abnormalities. Detailed descriptions of these studies were published in the February 2002 *Communiqué*.¹

Genetic Abnormalities in Plasma Cell Proliferative Disorders

Many genetic abnormalities have been specifically associated with multiple myeloma and other PCPDs (Table 1). Common abnormalities include translocations, numeric abnormalities, deletions, and point mutations, which can occur individually or in concert. When considered in the context of other disease features, these

Abnormality	Gene(s)/Region(s)	Prognostic Significance	Other Information
Aneuploidy			
- Hyperdiploid	N/A	Favorable	Common trisomies: 3, 5, 7, 9, 11, 15, 19, 21
- Near-tetraploid	N/A	Unfavorable	
Monosomy 13	Predominantly 13q14	Unfavorable	85% monosomy, 15% deletion
<i>IGH</i> Translocations			Uncommonly <i>IGL</i> (immunoglobulin light chain) translocations
- t(4;14)(p16.3;q32)	<i>FGFR3/MMSET</i>	Unfavorable	
- t(11;14)(q13;q32)	<i>CCND1</i>	Favorable	
- t(14;16)(q32;q23)	<i>c-maf</i>	Unfavorable	
- other <i>IGH</i> translocations	<i>CCND3, mafB, MUM1</i> , etc.	Unknown	
p53 inactivation	17p13	Unfavorable	Deletion or point mutation
<i>ras</i> mutations	<i>K-ras, N-ras</i>	Unfavorable (<i>K-ras</i>)	
Rb pathway inactivation	P16/ <i>INK4a</i> , P18/ <i>INK4c</i> , <i>RB1</i>	Unknown	
<i>PTEN</i> inactivation	<i>PTEN</i>	Unknown	
<i>c-myc</i> abnormalities		Unknown	

Table 1. Prognostic Significance of Common Genetic Abnormalities in Multiple Myeloma.⁵

genetic abnormalities can help define prognosis for patients with PCPDs. In general, patients with actively dividing plasma cells, and thus identifiable chromosome abnormalities, are more likely to have aggressive disease than those without chromosome abnormalities. Over time, recurrent cytogenetic anomalies have been identified that show specific prognostic significance in PCPDs.⁵

Normal cells contain 46 chromosomes. Aneuploidy, the simple gain or loss of whole chromosomes, is one common cytogenetic abnormality in PCPDs, and is most commonly observed as hyperdiploidy or near-tetraploidy. Hyperdiploidy, which refers to cells with >47 chromosomes, is associated with a favorable prognosis. Nearly half of myeloma patients have hyperdiploidy, with a median chromosome number of 53. Five to 10% of patients with myeloma have near-tetraploidy, which refers to cells with 75 or more chromosomes, and portends an unfavorable prognosis.⁵

Monosomy (and occasionally deletion) of chromosome 13 is the most common chromosome abnormality in PCPDs and appears to be an unfavorable prognostic marker when identified. Deletion of the tumor suppressor gene TP53 (p53) on the short arm of chromosome 17 is also associated with an unfavorable prognosis. In addition, this gene and several others (Table 1) can be inactivated through point mutations, which cannot be detected by routine cytogenetic or fluorescence in situ hybridization (FISH) techniques.⁵

More complex chromosome abnormalities are also observed, typically involving a translocation between 2 chromosomes. The most common translocations involve the immunoglobulin heavy chain gene (*IGH*). The prognostic implications of *IGH* translocations are dependent upon the translocation partner involved (Table 1). Several gene regions have been well characterized as translocation partners, while others have been less commonly documented, thus their prognostic implications are not as clear.⁵

While the chromosome abnormalities commonly identified in myeloma patients have also been demonstrated in patients with other PCPDs, the significance of these abnormalities is only known in patients with myeloma.⁶ Furthermore, identification of these abnormalities in myeloma precursors does not appear to predict in which patients the disease will evolve to myeloma.⁶

Novel FISH Testing Strategy

Historically, testing for genetic abnormalities in multiple myeloma has relied on conventional chromosome analysis. Chromosome studies are often useful at diagnosis if an abnormal clone is detected, as this indicates active disease. However, most bone marrow chromosome studies in PCPD patients are falsely normal since the actively dividing myeloid cells are analyzed rather than the plasma cells, which infrequently enter mitosis. Consequently, standard FISH testing (which does not require dividing cells for analysis) has more recently been employed to identify the classic abnormalities associated with myeloma. This method utilizes fluorescently labeled DNA probes that hybridize to specific chromosome regions. Since the cell cytoplasm is removed, many hundreds of nuclei are typically analyzed without the ability to distinguish between cell types. Therefore, like conventional chromosome studies, standard FISH testing can lead to an erroneously normal result due to low levels of plasma cells in blood and bone marrow. Relying solely on conventional chromosome analysis and standard FISH testing would result in the failure to detect abnormal PCPD clones in many cases. Thus, a better strategy for detecting the prognostically significant chromosome abnormalities was required.

We have developed a novel plasma cell-specific FISH method for detecting the known, prognostically significant chromosome anomalies in myeloma ([#83358 Plasma Cell Proliferative Disorder \[PCPD\], Fluorescence In Situ Hybridization \[FISH\]](#)). This new method, first

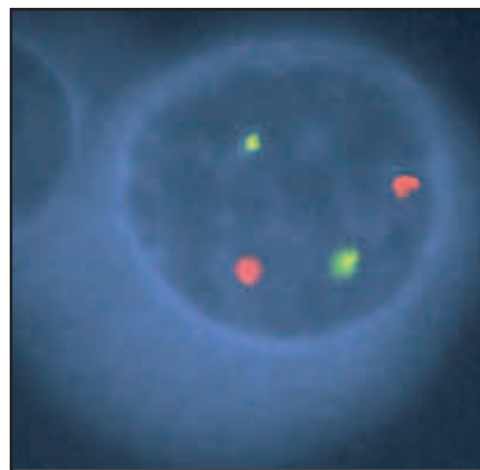


Figure 2. Normal plasma cell demonstrating 2 red and 2 green signals, specifically the p53 gene region at 17p13.1 (red) with 17 centromere (green) as a control.

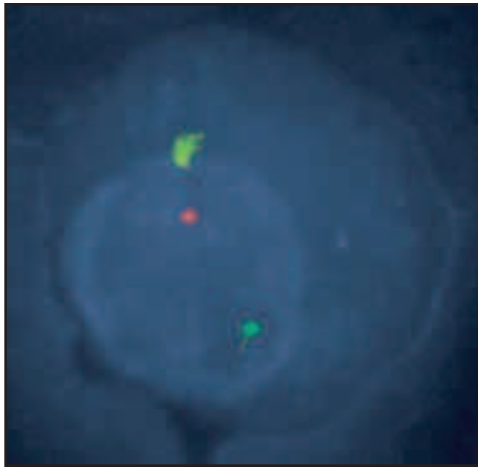


Figure 3A. Abnormal cell demonstrating deletion of the p53 gene region from chromosome 17. This signal pattern includes two 17 centromere (green) and one p53 (red) signal, indicating the loss of the p53 gene region.

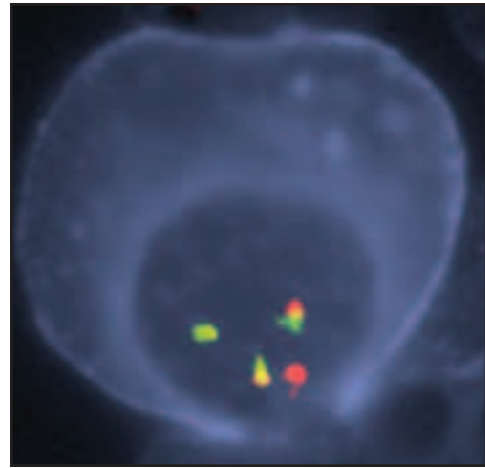


Figure 3C. Abnormal cell demonstrating fusion products from t(11;14)(q13;q32). This signal pattern includes 1 copy of *CCND1* (red), 2 copies of *IGH* (green), and 1 fusion product (yellow); representing 1 normal chromosome 11 (green), 1 normal chromosome 14 (red), and the abnormal chromosomes 11 and 14 involved in the translocation (yellow). The yellow signals are the result of the red and green signals relocating next to each other.

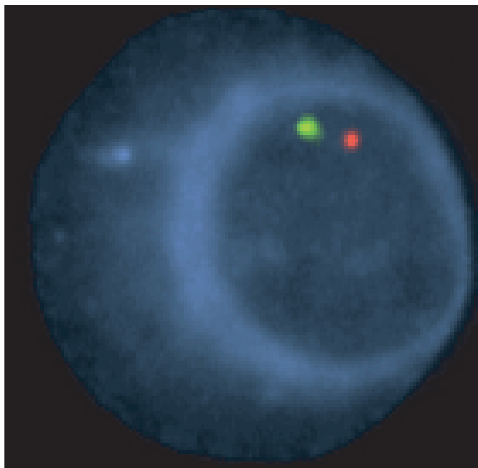


Figure 3B. Abnormal cell demonstrating only 1 red and 1 green signal. This signal pattern demonstrates the loss of 1 copy of the Rb-1 gene region (13q14, red) and the *LAMP1* gene region (13q34, green) and implies the presence of only 1 entire chromosome 13 (monosomy 13).

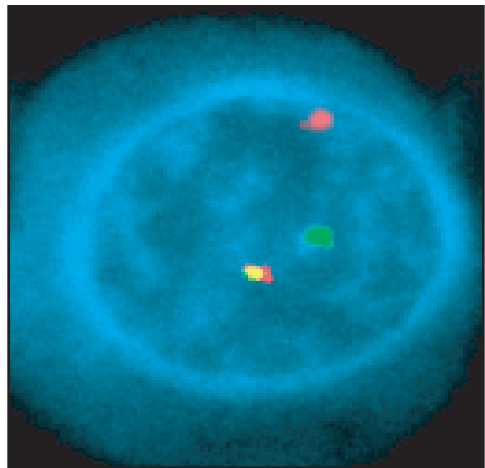


Figure 3D. Breakpart of 1 *IGH* copy into 3' (red) and 5' (green) ends of the gene. The normal copy of the gene is indicated by the yellow fusion signal. Breakpart of *IGH* in the absence of a t(11;14)(q13;q32) initiates reflex to FISH for t(4;14)(p16.3;q32) and t(14;16)(q32;q23).

Chromosome Abnormality	Gene Region/Control	FISH Strategy
Numeric abnormalities of 3, 7, 9, 15*	<i>CEP3, CEP 7, CEP 9, CEP 15</i>	Enumeration
-13/13q-	<i>RB1/LAMP1</i>	Enumeration
t(11;14)(q13;q32)	<i>CCND1;IGH</i>	Fusion
17p-	<i>p53/D17Z1</i>	Enumeration
14q32	<i>IGH</i>	Breakapart
t(4;14)(p16.3;q32)**	<i>FGFR3;IGH</i>	Fusion
t(14;16)(q32;q23)**	<i>IGH;c-maf</i>	Fusion

* Testing for hyperdiploidy is currently in validation but will be available as part of the cIg FISH panel in the near future.

** If 14q32 breakapart is abnormal and t(11;14) is not present, these 2 tests are performed.

Table 2. cIg FISH Panel for Multiple Myeloma

developed as a research tool, employs cytoplasmic antibodies to target plasma cells, in contrast with standard FISH methods that are unable to distinguish specific cell types. Because each plasma cell is restricted to expressing either κ or λ antibodies, anti- κ or anti- λ antibodies are used to tag the cytoplasmic immunoglobulin of plasma cells, depending on the light-chain restriction of a specific patient's disease.

The test is performed in 2 steps. First, FISH probes are hybridized to all cell types. Second, antibodies to the cytoplasmic immunoglobulins κ or λ are applied to specifically identify the plasma cells, causing the cytoplasm of these cells to fluoresce (Figure 1, page 1). Next, only plasma cells are scored for the panel of FISH probes targeted at the classic cytogenetic abnormalities seen in myeloma (Table 2, Figures 2 and 3). This process is called cytoplasmic immunoglobulin FISH, or cIg FISH.

The ability to target a specific cell type is only 1 characteristic that makes this technique novel as compared to classical FISH techniques. In standard FISH studies, the cytoplasm is stripped from the nucleus prior to applying the FISH probe. In contrast, the cytoplasm remains intact in cIg FISH testing so the appropriate anti- κ or anti- λ antibodies can be applied to identify the plasma cells. Also, in standard FISH testing, high numbers of nuclei (commonly between 200 and 500) are routinely scored because the entire cell population is included in the analysis. Using cIg FISH to specifically target plasma cells, smaller numbers of cells can be analyzed and still provide useful information, because only the cell type involved in the disease is being analyzed. Although it is ideal to

perform such a test when a large percentage of plasma cells exist in the bone marrow, cIg FISH for plasma cell proliferative disorders is capable of detecting abnormalities in bone marrow specimens even when few plasma cells are present. This test is performed on bone marrow samples rather than blood due to the lack of significant numbers of circulating plasma cells in blood specimens. Because cytoplasm is required for this technique, a fresh bone marrow specimen is required.

Method Comparison

In studies performed while validating the utility of cIg FISH, we compared the cIg FISH results with both conventional chromosomes and standard FISH analysis using probes for -13/13q- and t(11;14). Of 46 specimens analyzed, only 10 (22%) were abnormal by conventional chromosome analysis and 26 (57%) were abnormal by standard FISH analysis. In contrast, cIg FISH yielded abnormal results in 45 (98%) specimens.⁷

Appropriate Use of Testing

FISH tests in hematologic disorders are routinely used for 4 purposes: diagnosis, prognosis, tracking clonal evolution, and tracking minimal residual disease. Many FISH tests fulfill several of these applications, while others have very specific uses. cIg FISH has been developed for the sole purpose of identifying prognostic markers in patients with myeloma. Thus, only markers with a known prognostic importance are utilized in this assay.

cIg FISH is not used to diagnose myeloma or other PCPDs. While the chromosome abnormalities identified by cIg FISH will be localized to plasma cells, the test cannot distinguish between the anomalies seen in myeloma versus other PCPDs. Clinical evaluation and other laboratory tests are required to determine the specific PCPD in patients.

cIg FISH is not recommended to track clonal evolution of a disease because only a subset of chromosome regions are tested and anomalies involving chromosome regions not covered by the FISH probes will be missed with this assay. In addition, until further studies determine the clinical significance of clonal evolution in myeloma, tracking disease evolution by cIg FISH would not provide useful information for clinicians.

Lastly, cIg FISH is of little value for minimal residual disease (MRD) testing. While very low levels of disease can be detected with this method, we do not quantitate the number of plasma cells in relation to the total cell population. We solely analyze plasma cells and thus do not provide data on true MRD in these patients. Other less expensive laboratory tests (eg, serum and urine protein electrophoretic studies, plasma cell labeling index studies) are more suited to track minimal residual disease or disease progression in these patients.

Since cIg FISH is designed to identify specific chromosome abnormalities with known prognostic implications, it is recommended to use this test only once per patient, preferably at diagnosis or soon thereafter. This allows the treatment to be appropriately tailored toward the prognostic factors identified. Once the prognostic factors are determined, there is no need to perform this test on follow-up specimens as the chromosome abnormalities detected by cIg FISH will typically remain in the patient's clonal cells for the duration of the disease. Although diagnosis is the most ideal time for testing, cIg FISH can be attempted on any bone marrow specimen in patients with a suspected PCPD.

Future Utility for cIg FISH in Plasma Cell Proliferative Disorders

While cIg FISH is specifically used for prognostic information in multiple myeloma, it certainly has the potential to expand into several other areas in the future. First, the prognostic significance of classic PCPD abnormalities in nonmyeloma PCPDs may become better known. Because this method is able to target plasma cells, the testing for other PCPDs should be

relatively simple to implement. Second, this method has the potential to uncover chromosome abnormalities not classically described, such as duplication, deletion, or amplification of the regions examined by the FISH probes. Like the commonly known chromosome abnormalities, any newly described abnormalities may also have prognostic significance. Last, when the issue of clonal evolution becomes better understood, this testing may also become appropriate for disease tracking.

Conclusion

We recommend the use of cIg FISH for patients with multiple myeloma to detect the known, prognostically significant chromosome anomalies associated with myeloma. This test should be performed only once per patient, as it is not recommended for use in tracking treatment response.

#83358 Plasma Cell Proliferative Disorder (PCPD), Fluorescence In Situ Hybridization (FISH) can be ordered from Mayo Medical Laboratories. To ensure appropriate testing and interpretation, a reason for referral is requested for each specimen, and if known, the κ or λ restriction. A fresh 1.0–2.0-mL bone marrow specimen in sodium heparin is required. Specimens should be shipped ambient, to ensure a viable specimen.

Any questions about specimen requirements or shipping instructions should be forwarded to Mayo Laboratory Inquiry at 800-533-1710.

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2. Grogan TM, et al: Plasma Cell Neoplasms, *In* Pathology and Genetics of Tumors of Haematopoietic and Lymphoid Tissues. Edited by ES Jaffe, et al. IARC Press, International Agency for Research on Cancer: Lyon, France, 2001, p 142-156
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7. Knudson RA, et al: Use of the cIg FISH method in Clinical FISH Testing for the Common Chromosome Abnormalities Associated with Plasma Cell Proliferative Disorders. *In press*

Test Updates

Mycobacterium tuberculosis Method Change

In conjunction with the introduction of the high-concentration streptomycin and isoniazid tests (see New Test Announcements #83676 and #83677), [#81309](#)

Antimicrobial Susceptibility, Mycobacterium tuberculosis Complex has changed from a radiometric/minimum inhibitory concentration method to the BACTEC MGIT 960. There are no other changes to the test.

Drugs of Abuse Urine Panel Changes

The fee structure for the Confirmed Drugs of Abuse urine panels will be changing to better serve our clients and patients. Historically, the fees per orderable drug abuse test, a panel of several screening tests coupled with confirmation of positives, have been cost averaged. Patient specimens that screen negative, requiring no confirmation testing, were priced the same as patient specimens that screen positive and have confirmatory tests performed. This resulted in significant cost savings for positive specimens, but caused negative reports to bear a disproportionate component of the confirmation cost associated with other specimens.

Confirmed Drugs of Abuse tests will now be charged in line with current market practice, ie, negative specimens will only incur the cost of screening and reporting. While billing will change for both positive and negative specimens, reporting will change only for positive specimens. When a positive screen result is ready to report, the screening results will be available immediately. The report will state: "Drug confirmation ordered by reflex. This result is presumptive. Refer to confirmation report for the definitive result." When the confirmation test is performed, it will be billed separately and a separate report will follow.

With these changes, clients will see their presumptive positive screening results quickly, and the separate billing will reflect additional charges for the individual positive components.

These changes will affect the following tests:

Test	Published Name
9446	Drug Abuse Survey with Confirmation, Urine
80917	Drug Abuse Survey with Confirmation, Evaluation 1, Urine
80918	Drug Abuse Survey with Confirmation, Evaluation 2, Urine
80919	Drug Abuse Survey with Confirmation, Evaluation 3, Urine
80373	Drug Abuse Survey with Confirmation, Panel 5, Urine
81410	Drug Abuse Survey with Confirmation, Evaluation 12, Urine

Specimens with positive screening results will reflex to the following tests, as appropriate:

Test	Published Name
8826	Volatile Screen, Urine (Alcohol)
8257	Amphetamine, Urine
80372	Barbiturate Confirmation, Urine
80370	Benzodiazepine Confirmation, Urine
9286	Cocaine Confirmation, Urine
81743	Lysergic Acid Diethylamide (LSD) Confirmation, Urine
83219	Methadone Confirmation, Urine
8587	Drug Screen, Urine (Methaqualone)
83128	Propoxyphene, Urine
8473	Opiates, Urine
80371	Phencyclidine Confirmation, Urine
8898	Tetrahydrocannabinol (THC) Confirmation, Urine

Ask



US

Please e-mail your questions to mml@mayo.edu.

Q: Why is the inborn errors of metabolism screen (**#8396 Inborn Errors of Metabolism Screen, Plasma and Urine**) being deleted? Which tests should be ordered instead of the screen?

A: **#8396 Inborn Errors of Metabolism Screen, Plasma and Urine** does not provide comprehensive screening for all inborn errors of metabolism, such as organic acidemias and fatty acid oxidation disorders. All of the tests included in the screen are now individually available either from your local laboratory or Mayo Medical Laboratories. By offering the individual assays, physicians may tailor testing for each patient according to the clinical presentation. The following IEM tests are available:

- #82413 Acylcarnitines, Quantitative, Plasma
- #83172 Amino Acids, Qualitative, Plasma
- #8400 Amino Acids, Qualitative, Urine
- #83661 Amino Acid Screening Profile, Plasma and Urine
- #9359 Biotinidase, Serum
- #9255 Carbohydrate, Urine
- #82414 Carbohydrate Deficient Transferrin, Serum
- #8802 Carnitine, Plasma or Serum
- #82042 Fatty Acid Profile, Comprehensive (C8-C26), Serum
- #81939 Fatty Acid Profile, Mitochondrial (C8-C18), Serum
- #81369 Fatty Acid Profile, Peroxisomal (C22-C26), Serum
- #9252 Homogentisic Acid, Urine
- #84464 Mucopolysaccharides Screen, Urine (available 1/18/05)
- #84340 Oligosaccharides Screen, Urine
- #80619 Organic Acids Screen, Urine
- #81420 Purine and Pyrimidine Panel, Urine
- #82079 Sterols, Plasma

If you need assistance determining appropriate testing for your patient, please contact Mayo Laboratory Inquiry at 800-533-1710 and ask to speak with a genetic counselor or consultant in the Biochemical Genetics Laboratory.

– *Continued on next page.*



Please e-mail your questions to mml@mayo.edu.

Q: Why does MML encourage ordering a von Willebrand disease (vWD) profile ([#554 Coagulation Consultation, von Willebrand Disease, Plasma](#)) for patients with suspected von Willebrand disease rather than a von Willebrand Factor (vWF) Multimer Analysis ([#8844 von Willebrand factor multimer analysis, Plasma](#))?

A: For patients with suspected vWD, MML recommends performing a vWD profile (#554) because it includes all of the tests necessary for diagnosing vWD (eg, ristocetin cofactor activity, vWF antigen level, factor VIII activity) and an interpretive report. Multimer analysis, which is only used to identify vWD type, is reflexively performed when appropriate.

von Willebrand disease is a relatively common bleeding disorder that manifests clinically as easy bruising, mucocutaneous bleeding (eg, epistaxis, menorrhagia, etc), and bleeding after trauma or surgery. Most vWD is inherited, although about 1% to 4% of patients have acquired vWD, usually due to lymphoproliferative or plasma cell proliferative disorders (eg, monoclonal gammopathies). Unlike hemophilia, vWD is an autosomal disorder and affects both men and women.

von Willebrand factor has 2 main functions: 1) vWF functions as a ligand by binding to the platelet receptor GPIb and to collagen, and 2) vWF functions as a carrier molecule for plasma procoagulant factor VIII. Type 2N (Normandy) vWD is a very rare inherited disorder causing reduced binding of factor VIII to vWF; these patients have mildly reduced plasma factor VIII activity (eg, ~15%) and normal ristocetin cofactor activity and vWF antigen level. Excepting this rare variant, all other vWD types have reduced ristocetin cofactor activity. The ristocetin cofactor activity assay is complex and best performed in an experienced laboratory using fresh donor platelets. A diagnosis of vWD requires evidence of both clinically abnormal bleeding as well as reduced ristocetin cofactor activity. Depending on the vWD type, the plasma vWF antigen level and factor VIII activity also may be reduced.

Q: When do Mayo hematologists order a plasma vWF multimer analysis ([#8844 von Willebrand Factor Multimer Analysis, Plasma](#))?

A: A plasma vWF multimer analysis is unnecessary for the diagnosis of vWD and should only be performed when the ristocetin cofactor activity and vWF antigen level are either abnormally low or the ristocetin cofactor activity: vWF antigen level ratio is discordantly low (eg, <0.7). The multimer analysis is only used to clarify the type of vWD (types 1, 2, 3, or acquired vWD).

A plasma vWF multimer analysis is reflexly performed as part of the vWD profile (test #554) when the above criteria are met. This has the advantage of avoiding collection of an additional blood sample for a multimer analysis should the screening tests for vWD (eg, ristocetin cofactor activity, vWF antigen level, factor VIII activity) prove to be abnormally low or discordant.

– Continued on next page.

Ask



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Please e-mail your questions to mml@mayo.edu.

Q: Is the vWF multimer analysis (#8844 von Willebrand Factor Multimer Analysis, Plasma) useful for diagnosing thrombotic thrombocytopenic purpura (TTP)?

A: No. A vWF multimer analysis is not useful for other purposes such as diagnosis of TTP or hemolytic-uremic syndrome (HUS).

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