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METHOD AND ORDER CODE CHANGES FOR SERUM PROTEIN ELECTROPHORESIS

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Effective August 3, 2015, capillary electrophoresis (CE) using the CAPILLARYS™ 2 Flex Piercing system by Sebia, Inc., will replace the current agarose gel method for serum protein electrophoresis performed by Marshfield Labs. Accordingly, some order codes related to protein electrophoresis will be modified.

BACKGROUND

Protein electrophoresis is used in clinical laboratories to evaluate serum samples for protein abnormalities, especially monoclonal gammopathies. Separation of serum proteins by CAPILLARYS™ system is achieved by the electrophoretic mobility of proteins in an alkaline buffer with a specific pH (9.9) and the electroosmotic flow of the buffer solution in the capillary. Samples are introduced at the anodic end of the capillary. Proteins are separated by applying a high voltage and detected directly at the cathodic end of the capillary photometrically at 200 nm. The electrophereogram is similar to that of the current gel system with five major zones: albumin, alpha1, alpha2, beta, and gamma. (Figure 1.)

The most significant difference between these two methods is in how they identify monoclonal proteins: subtraction immunotyping (IT) by CAPILLARYS versus immunofixation electrophoresis (IFE) by the agarose gel system.

The identification of monoclonal protein by IT is achieved by adding antibodies directed against IgG, IgA, IgM, kappa, and lambda proteins to the sample serum thereby forming immune complexes that migrate anodically. These complexes remove both

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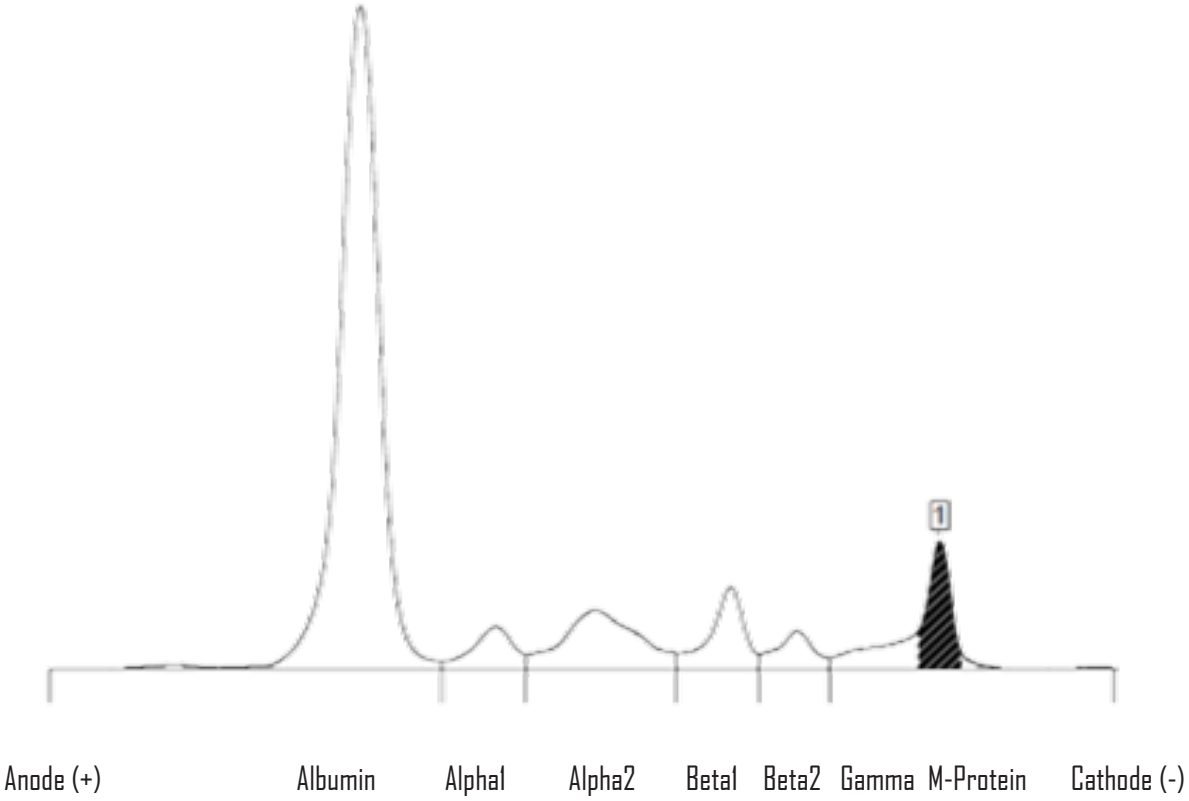
monoclonal and polyclonal immunoglobulins. Each antiserum pattern is overlaid with the original protein electrophoresis curve. Disappearance of the abnormality in the antiserum-treated pattern indicates the presence of a monoclonal protein. (Figure 2.)

Figure 1. CAPILLARYS Electrophoretic Pattern



WHAT TO EXPECT

- New reference intervals for all proteins: UV absorbance detection vs dye-protein binding staining densitometry.
    - Alpha-1 globulins are increased due to the high concentration of sialic acid in alpha1-acid glycoprotein that is insensitive to dye.
- The reference curves by CE are slightly more sensitive in detecting monoclonal proteins.
- IT is slightly less sensitive than IFE in detecting low-concentration monoclonal immunoglobulins (<0.1 g/dL), particularly IgM clones, and low-level free light chains such as those often observed in patients with monoclonal gammopathy of undetermined significance (MGUS). Since the detection of these very low-level monoclonal proteins is rarely clinically significant, this is not a major drawback.
- No changes in the interpretive comments.
- No CPT code changes.

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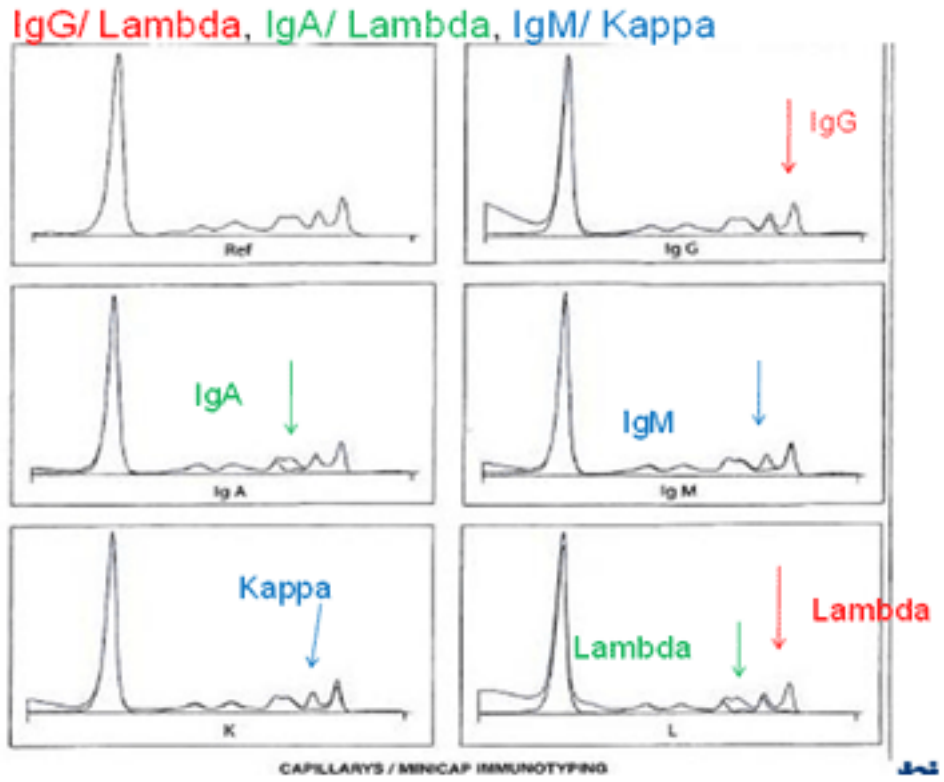
Figure 2. Example of Immunotyping (IgG/Lambda, IgA/Lambda, and IgM/Kappa)



TEST INFORMATION

Test Name	*Test Code*	*Specimen Type*
Protein Electrophoresis	PEP	Serum
Immunoglobulins G-A-M & Immunotyping add-on	GAM-IT	Serum
Protein-Immunotyping (PEP, GAM, IT) Panel	IEPIT	Serum
Protein-Immunotyping (PEP, GAM, IT) and Beta 2 Microglobulin	GAMPRIT	Serum

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REFERENCE INTERVALS

Protein	New Reference Interval (g/dL) by Capillary Electrophoresis	Previous Reference Interval (g/dL) by Agarose Gel
Albumin	3.7 – 4.9	3.80 – 5.00
Alpha 1	0.2 – 0.4	0.12 – 0.22
Alpha 2	0.5 – 0.9	0.52 – 0.82
Beta	0.6 – 1.0	0.53 – 1.00
Gamma	0.6 – 1.4	0.40 – 1.20

QUESTIONS

Test information is available in Marshfield Labs' Test Reference Manual.
For Clinical and Technical information contact:

- Joyce L. Flanagan, Ph.D. Clinical Chemist.
- Thomas Schulta, Assistant Manager of Clinical Esoteric Manual Section.
- Phone number: 800-222-5835.

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1. Keren DF. *Protein Electrophoresis in Clinical Diagnosis*. American Society for Clinical Pathology Press; 2012.
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3. Chartier C, Boularan AM, et al. *Evaluation of two automated capillary electrophoresis systems for human serum protein analysis.* Clin Biochem. 2011; 44:1473-1479.
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NEW DIAGNOSTIC CASCADE FOR HEPARIN INDUCED THROMBOCYTOPENIA

Gene R. Shaw, MD, Clinical Pathology

Effective August 3, 2015, Marshfield Labs will offer a diagnostic cascade for the laboratory evaluation of possible heparin induced thrombocytopenia (HIT). This approach will optimize turn-around-time by using a highly sensitive in-house platelet factor 4 (PF4) ELISA test with equivocal or positive results to be followed by a more specific serotonin release assay (SRA) performed by the Blood Center of Wisconsin. Based on data gathered over the past several months, 93% of PF4 assays are negative and unless the clinical situation is virtually classic for HIT, a negative PF4 essentially excludes the diagnosis. Individually ordering the PF4 test or SRA as stand-alone tests will remain available for outreach accounts, but requires pathologist approval within the Marshfield system.

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BACKGROUND

HIT is a rare but serious complication of heparin therapy. Patients suspected of having HIT often have a very complex medical situation with a variety of other possible causes for thrombocytopenia and/or thrombosis. Furthermore, argatroban (the direct thrombin inhibitor most commonly used in HIT) may cost over $1000/day and carries a risk of bleeding that is not readily reversed. Timely laboratory testing plays a pivotal role in correctly diagnosing these patients.[1,2] The pretest probability (e.g., using scoring systems like the 4Ts) is critical for immediate patient management and putting these lab results in context.[3]

In reviewing test utilization patterns, it has become apparent that clinicians may be inappropriately ordering lab tests in the work-up of HIT. The excellent negative predictive (rule-out) value of the platelet factor 4 (PF4) IgG test is often not appreciated and too many providers are requesting SRA testing. Note that the SRA is widely regarded as the best available test for HIT, but using radioactive-labeled serotonin, is only available at a few labs across the USA. The Blood Center of WI does most of the SRA testing for the Upper Midwest.

Review of 102 consecutive PF4 tests over a 4-month period showed that the vast majority (93%) were negative (optical density < 0.400). Chart review was performed on the remaining seven equivocal or positive tests. Only one test was a high-level positive (OD 2.571) which was confirmed by SRA. This patient had clinically evident HIT. In the remaining 5 patients (6 tests as one patient was tested twice during the interval of this look-back) the OD was between 0.400 and 1.000. None had compelling clinical or laboratory evidence of HIT (3 had negative SRA testing; in 2 it wasn't done). In 4 of the 5 patients, the "confirmatory" inhibition step with excess heparin was positive showing its lack of utility in this situation. Further review of the literature also casts significant doubt on the utility of this "confirmation" step and it is not used by some large reference laboratories. The 50% inhibition criterion appears to be arbitrary. One study cited to support its use did not do parallel SRA testing, did not use an IgG-specific PF4 assay, and used admittedly controversial clinical criteria as their "gold standard" for classifying patients.[4] In 2010 Marshfield Labs converted to an IgG-specific EIA from Immucor that purportedly retains the sensitivity of the prior GAM assay while improving specificity.

Based on this information the following changes will be implemented:

A new lab test will be offered called Heparin-Induced Thrombocytopenia (HIT) Diagnostic Cascade. A serum sample will initially be tested using the Immucor IgG-specific platelet factor 4 (PF4) enzyme immunoassay. The excess heparin "confirmatory" step will be dropped which incidentally will result in nominal reductions in reagent costs and technical time. The vast majority of samples with results less than 0.400 optical density (OD) will simply be reported as negative. Results 0.400 OD and greater will be reported in two tiers (0.400 to 1.000 OD and > 1.000 OD, respectively, and the remaining specimen will be forwarded to The Blood Center of WI for the SRA which is done daily at that facility. Updated interpretative comments are provided below:

- < 0.400 OD: NEGATIVE. This result has approximately 90-95% negative predictive value for excluding heparin-induced thrombocytopenia (HIT).

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• 0.400-1.000: EQUIVOCAL. Further testing with the serotonin release assay (SRA) to be performed by The Blood Center of WI should be available within 1-2 days. Most results in this range are not confirmed by SRA. Also, consider repeat testing if the clinical situation remains unclear.

• > 1.000: POSITIVE. Heparin dependent antibodies are present. This result has a reasonably good correlation with the serotonin release assay (SRA) to be performed by The Blood Center of WI which should be available within 1-2 days. If the clinical situation warrants, initiating treatment for heparin-induced thrombocytopenia (HIT) is reasonable.

As stand-alone tests, the PF4 test and SRA will only be available by pathologist approval within the Marshfield system.

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KRAS MUTATION ANALYSIS TEST (KRAS) AND *NRAS* MUTATION ANALYSIS TEST (NRASSO) DISCONTINUED

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Bruce Krawisz, MD, Anatomic Pathology

Effective immediately, Marshfield Labs has discontinued the *KRAS* Mutation Analysis Test (Test Code: KRAS) and the *NRAS* Mutation Analysis test (Test Code:NRASSO).

The primary role for this test has been to guide therapy in cases of metastatic colorectal cancer (mCRC). New discoveries have led to an expansion of relevant targets beyond the *KRAS* gene. The 2015 National Comprehensive Cancer Network Colon Cancer Guidelines now recommend an "extended RAS/RAF panel" to include interrogation of *KRAS* and *NRAS* (exon 2 and non-exon 2) mutations as well as the *BRAF* V600E mutation.[1] On April 22, 2015, the American Society for Clinical Pathology (ASCP), the College of American Pathologists (CAP), the Association for Molecular Pathology (AMP), and the American Society of Clinical Oncology (ASCO) closed the public comment period on their draft of a clinical practice guideline for the use of molecular marker testing for patients with primary or metastatic colorectal carcinoma. This draft guideline explicitly called for interrogation of exons 2, 3 and 4 of both the *KRAS* and *NRAS* genes.[2] The final guidance document is expected to be published by the end of the year.

Neither the KRAS nor the NRASSO tests interrogate the entire spectrum of mutations now recommended to guide therapy for mCRC. We recommend Test ID: "RASFPSO

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RAS/RAF Targeted Gene Panel by Next Generation Sequencing, Tumor" offered by Mayo Laboratories. This test interrogates mCRC relevant mutations in the *KRAS*, *NRAS*, *HRAS* and *BRAF* genes. This test should be ordered for any mCRC tumor sample when guidance is sought regarding the use of an EGFR inhibitor for treatment. This test code can be submitted on a miscellaneous test request form with the test code above and the note please send to Mayo.

QUESTIONS

Test information is available in Marshfield Labs' Test Reference Manual.
For clinical information contact:

- Bruce Krawisz, MD; or Timothy Uphoff, PhD.
- Phone number: 800-222-5835.

REFERENCES

1. National Comprehensive Cancer Network. Colon Cancer (Version 2.2015). https://www.nccn.org/store/login/login.aspx?ReturnURL=http://www.nccn.org/professionals/physician_gls/pdf/colon.pdf . Accessed June 24, 2015.

2. ASCP/CAP/AMP/ASCO Evaluation of Molecular Markers for Colorectal Cancer Draft Recommendations. http://www.amp.org/committees/clinical_practice/CRCOpenComment.cfm. Accessed June 24, 2015.