LAB FOCUS

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Molecular Diagnostics Laboratory offers tests for chronic myeloproliferative disorder, renal coloboma syndrome and warfarin dosing.

Test for fusion gene BCR/ABL helps monitor patients with chronic myeloproliferative disorder (CML).

Chromosomal translocation (9;22) leads to a fusion gene BCR-ABL—a signature feature of chronic myeloproliferative disorder (CML). During and after treatment, providers can use regular RT-PCR to monitor patients with CML for the presence or absence of BCR-ABL fusion transcripts. But while regular RT-PCR can detect the fusion gene transcripts, it is not appropriate for the detection of tumor load and is not accurate for detection of minimal residual disease after treatment.

Quantitative RT-PCR, however, can provide important information to monitor the tumor load, status of remission, minimal residual disease, and chronological expression level of the fusion transcripts.

The molecular lab is offering the Real Time Quantitative PCR (qRT-PCR) assay for BCR-ABL1 fusion transcripts, p210(major breakpoint of BCR-ABL1), for CML only. This test does not include BCR/ABL1 fusion transcripts, p190 (minor breakpoint of BCR-ABL1), for ALL (acute lymphoblastic leukemia). Physicians should provide the same specimen type, preferably blood, for follow-up testing for BCR-ABL CML. The laboratory will accept bone marrow samples, but such samples will not be included in cumulative results graph.

The qRT-PCR assay includes reverse transcription of extracted RNA and real time PCR using TaqMan® technology. TaqMan technology uses a pair of PCR-primers to amplify the breakpoint region of BCR-ABL fusion gene and uses the TaqMan probe to specifically detect both b2a2- and the b3a2-variants of BCR-ABL fusion transcripts.

The laboratory can detect exact copy number of fusion transcripts in each sample and report the ratio of the fusion transcripts over transcripts of normal ABL gene. The lab will report the variation of ratios from the same patient chronologically.

Michelle Dolan, M.D.
Xinjing Wang, Ph.D.
Teresa Kemmer, C.L.S.

Matt Schomaker, Clinical Laboratory Scientist, tests for the fusion gene BCR/ABL—a signature feature of chronic myeloproliferative disorder (CMD).

PAX2 gene sequencing aids screening, diagnosis of renal coloboma syndrome.

Mutations in the PAX2 gene cause renal coloboma syndrome, an autosomal dominant disease characterized by renal abnormalities and colobomatous eye defects. Typical findings include renal hypoplasia, vesicoureteric reflux, renal insufficiency and optic disc coloboma. Less than 20 percent of patients also experience high frequency hearing loss, Arnold Chiari malformation, seizures and joint laxity.

Genetic screening for PAX2 mutation allows for molecular confirmation of renal coloboma syndrome in about 50 percent of affected individuals, including prenatal target mutation analysis and differential diagnosis of coloboma and/or renal hypoplasia.

The literature has reported mutations in the PAX2 gene in more than 50 individuals with renal coloboma. Seventeen different PAX2 mutations have been identified, including nucleotide substitutions, insertions and deletions—and a single reported case of de novo translocation.

The paired box (PAX) genes are a family of developmental control genes. PAX2 gene is one of nine PAX genes located

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on human chromosome 10q24-25 and comprised of 12 exons spanning approximately 84 kb of genomic DNA. The PAX2 gene encodes for a 1.3kDa protein which is important in the development of the kidney, eye, inner ear, urogenital tract and central nervous system, and is involved in regulating such genes as WNT4, WT1, N-myc, and p53.

The molecular laboratory has developed a sequencing assay to detect mutations in the coding regions of the PAX2 gene. Amplification of genomic DNA uses sequence-specific primers linked to M13 forward or reverse primers. DNA sequencing uses Big Dye terminator technology, while the M13 tails function as universal primers. Staff performs the analysis on an ABI 3100-Avant Genetic Analyzer.

For more information about either test, contact the laboratory, 612-273-8445.

Michelle Dolan, M.D.
Xinjing Wang, Ph.D.
Teresa Kemmer, C.L.S.

Warfarin sensitivity by CYP2C9 & VKORC1 genotyping helps accurate dosing.

Warfarin overdosing and underdosing can result in such life-threatening events as bleeding or thrombosis. Standard dosing algorithms do not account for individual variation in warfarin sensitivity. As a result, more than one third of over-anticoagulatted patients remaining outside their target INR 24 hours after treatment. Such patients are at increased risk for bleeding complications.

Variation in warfarin sensitivity depends on such genetic and non genetic factors as age, concurrent medication use, body weight, co-morbidity, frailty, warfarin daily dose, etc. Variants in the CYP2C9 and VKORC1 genes have been associated with the differences in the maintenance warfarin dose and an increased risk of bleeding after initiation of warfarin therapy. The common CYP2C9 variants (*2 and *3) with the VKORC1 promoter mutation (c.-1639G>A), are estimated to account for approximately 30-35 percent of the variability in therapeutic warfarin dose.

Laboratory staff uses a multiplex, primer extension based assay, Mutector II. Mutector II uses a specially modified enzyme mixture and unique synthetic terminators to distinguish between the normal and variant alleles by extending the detection primers with multiple bases in a sequence-specific manner. The extended primers are then sorted on a sequencer (ABI 3100 Genetic Analyzer) and the specific alleles are identified by fragment analysis.

The assay detects only the two common CYP2C9 variants, CYP2CP *2 (c.-430C>T) and CYP2C9 *3 (c.-1075A>C), and one common VKORC1 variant (c.-1639G>A). It does not detect other variants that may impact warfarin sensitivity or resistance in these or other genes.

Bharat Thyagarajan, M.D.
Xinjing Wang, Ph.D.
Teresa Kemmer, C.L.S.

Cyclic citrullinated peptide antibody testing aids diagnosis of rheumatoid arthritis

To aid in the diagnosis of rheumatoid arthritis, the Protein Laboratory at University of Minnesota Medical Center, Fairview began testing for antibodies to cyclic citrullinated peptide (CCP) by Enzyme-Linked ImmunoSorbent Assay (ELISA or enzyme immunoassay) in May.

Clinical Background: Rheumatoid arthritis (RA) is a chronic debilitating disease characterized by inflammation of the lining of the joints. It can lead to long-term joint damage, resulting in chronic pain, loss of function and disability. While the cause is unknown, early diagnosis and treatment are important to minimize the damage and complications.

Auto-antibodies to CCP appear early in the course of RA and are found in 60-80 percent of RA patients. This is comparable to the sensitivity of the rheumatoid factor test (RF). However, anti-CCP is more specific for RA (98 percent) and, unlike RF, rarely is found in diseases other than RA. Moreover, CCP antibodies may be present in patients with a negative RF.

Interpretive Information: The concurrent presence of anti-CCP increases the predictive value of RF. The presence of CCP antibodies is associated with a greater tendency toward the more destructive forms of RA. The diagnostic value of the presence of CCP antibodies in juvenile rheumatoid arthritis patients has not been determined.

Reference range:
< 5 U/mL = Negative,
> 5 U/mL = Positive for anti-CCP antibodies

Michael Steffes, M.D., Ph.D., medical director,
Special Chemistry Laboratory
Linda Bailey, C.L.S., technical supervisor