A Special Article on guidelines for detecting Janus kinase 2 (JAK2) and myeloproliferative leukemia virus oncogene (MPL) mutations in myeloproliferative neoplasms, a Technical Advance on the molecular diagnosis of congenital adrenal hyperplasia, and research articles on the development of a second-generation sequencing assay for the detection of hereditary BRCA1/BRCA2 mutations and the isolation and stability of circulating miRNAs in plasma were selected for the November 2013 JMD CME Program in Molecular Diagnostics. The authors of the referenced articles and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.


Questions #11-12 are based on: Sourvinou IS, Markou A, Lianidou ES: Quantification of circulating miRNAs in plasma: Effect of preanalytical and analytical parameters on their isolation and stability. J Mol Diagn 2013, 15:827-834; http://dx.doi.org/10.1016/j.jmoldx.2013.07.005

Upon completion of this month’s journal-based CME activity, you will be able to:

- Discuss the Janus kinase 2 (JAK2) and myeloproliferative leukemia virus oncogene (MPL) mutations found in polycythemia vera (PV) and thrombocythemia (ET).
- Understand the diagnostic value of JAK2 and MPL mutations in myeloproliferative neoplasms (MPN).
- Describe the structure of the JAK2 and MPL genes.
- Describe the methods used to detect JAK2 and MPL mutations.
- Discuss congenital adrenal hyperplasia (CAH).
- Understand the role of CYP21A2 mutations for the detection of CAH.
- Describe the importance of detecting BRCA1 and BRCA2 mutations in breast and ovarian cancers.
- Discuss sequencing methods used by clinical laboratories to examine gene mutations, including BRCA1 and BRCA2.
- Understand the role of circulating miRNAs in plasma and their stability when isolated and stored.
1. Recurrent mutations in the Janus kinase 2 (JAK2) gene and the myeloproliferative leukemia virus oncogene (MPL) are genetic hallmarks of BCR-ABL1 – negative myeloproliferative neoplasms (MPN). Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:733-744.]
   a. A point mutation resulting in a stop codon in the tyrosine kinase gene JAK2 in BCR-ABL1 – negative MPN was first described in 2003.
   b. A JAK2 mutation in codon 617 was found in the vast majority of patients with polycythemia vera (PV) and in approximately half of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF).
   c. In 2007, mutations in exon 12 of the JAK2 gene were found in a small percentage of PV patients.
   d. Exon 12 mutations and the V617F mutation are mutually exclusive.

2. Laboratory tests for JAK2 and MPL have become standard in assessing clinically suspected BCR-ABL1 – negative MPN. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:733-744.]
   a. Mutations in MPL were identified in both ET and PMF patients in 2006.
   b. MPL mutations are not present in JAK2 mutation-positive cases.
   c. The 2008 World Health Organization classification of hematopoietic neoplasms includes JAK2 mutations as diagnostic criteria in PV and JAK2 and MPL mutations in ET and PMF.
   d. A diagnosis of PV can be made when JAK2 V617F or exon 12 mutation is detected, along with increased hemoglobin and low or normal levels of erythropoietin.

3. The JAK2 gene maps to chromosome band 9p24 and encodes a tyrosine kinase protein composed of 1132 amino acids. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:733-744.]
   a. The JAK2 gene contains three critical domains: JH1, JH2, and four-point-one, ezrin, radixin, moesin homolog domains.
   b. JH1, the catalytic phosphokinase domain, is located at the carboxyl terminus and induces phosphorylation of target proteins.
   c. JH2 is structurally similar to JH1 but functions as a pseudokinase domain that negatively regulates basal activity of the kinase domain and receptor-induced activation of the catalytic function.
   d. The most frequent exon 12 mutation is an in-frame insertion of three nucleotides at codon 542.

4. The MPL gene maps to chromosome band 1p34 and encodes the thrombopoietin receptor, which binds to thrombopoietin. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:733-744.]
   a. Binding of thrombopoietin to MPL leads to activation of JAK2, which phosphorylates MPL and initiates a cascade of downstream signaling events that regulate cell survival, proliferation, and differentiation.
   b. Mutations of the MPL gene occur in BCR-ABL1 – negative MPN.
   c. The majority of the MPL mutations are found in exon 10 codon 515.
   d. MPL W515L or W515K mutations are present in patients with PMF and ET at a frequency of approximately 2% and 4%, respectively.

5. A number of methods have been developed for detecting JAK2 V617F mutation. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:733-744.]
   a. The most widely used methods for the detection of JAK2 V617F mutation involve allele-specific qPCR.
   b. Allele-specific qPCR achieves analytical sensitivities of ≤1% mutant alleles.
   c. Allele-specific qPCR allows for the quantification of the mutant as a percentage of all of the JAK2 alleles, as an estimate of disease burden.
   d. The clinical utility of quantification of JAK2 V617F has clearly been established.

6. Initial testing of JAK2 and MPL mutations is most commonly performed on peripheral blood samples. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:733-744.]
   a. Total white blood cells from the peripheral blood are the preferred type of cell population for JAK2 and MPL mutation analyses.
   b. The allele burden varies greatly (between 1% and 100%) from patient to patient at the time of first diagnosis of PV, and low levels of JAK2 V617F are not uncommon.
   c. With quantitative assessment, it has been reported that approximately 50% of PV patients have less than 25% JAK2 V617F alleles.
   d. A high allele burden in PV and ET is associated with progression to myelofibrosis.
7. Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:745-753.]

- a. CAH is manifested in a variety of clinical severities, comprising three subtypes: classic salt wasting, classic simple virilizing, and nonclassic forms.
- b. The incidence of classic CAH worldwide ranges from 1 in 5,000 to 1 in 15,000 births.
- c. CAH is characterized by impairment of cortisol biosynthesis, with or without impairment of aldosterone biosynthesis.
- d. About 95% of CAH cases are due to 21-hydroxylase deficiency (21-OHD).

8. The steroid 21-hydroxylase gene, CYP21A2, is located in the HLA class III region on chromosome 6p21.3. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:745-753.]

- a. Molecular analysis of CYP21A2 is of great importance to understanding the etiology of 21-OHD.
- b. Large CYP21A2 gene rearrangements have been traditionally detected by Southern blot analysis.
- c. Approximately 25% to 30% of CYP21A2 mutations observed in CAH are deleterious mutations derived from pseudogene CYP21A2P due to small gene conversions.
- d. Recently, multiplex ligation-dependent probe amplification has been increasingly used for identification of CYP21A2 gene deletion/duplication.

9. Individuals who inherit mutations in BRCA1 or BRCA2 are predisposed to breast and ovarian cancers. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:796-809.]

- a. Germline mutations in BRCA1 and BRCA2 are implicated as causal factors in up to 15% of all breast cancers diagnosed annually.
- b. Testing for these mutations is an important tool to identify individuals who would benefit from prophylactic surgery or increased breast surveillance.
- c. The relatively large size of the BRCA1 and BRCA2 genes and large number of patient referrals create a burden on genetic testing resources, affecting costs and wait times.
- d. The demands placed on clinical genetics laboratories will be compounded as additional genes are associated with predisposition to disease.

10. Currently, genes are sequenced in the vast majority of clinical laboratories using the dideoxy Sanger method coupled with capillary electrophoresis. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:796-809.]

- a. The data characteristics and the accuracy and sensitivity of dideoxy sequencing are well defined.
- b. The Sanger method is well suited to the detection of single-base substitutions, small insertions and deletions, as well as larger copy-number changes.
- c. Second-generation sequencing technologies have substantially changed the scale and efficiency of DNA sequencing.
- d. Second-generation sequencing technologies have redefined the scope of what can practically be interrogated in a clinical sequencing assay.

11. miRNAs are a class of small, endogenous, noncoding, single-strand RNAs. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:827-834.]

- a. miRNAs can negatively regulate gene expression by binding to specific complementary sites at the 3’ untranslated region of target mRNAs, causing translational repression or transcript degradation.
- b. Although miRNAs act as oncogenes, they do not function as tumor suppressor genes.
- c. miRNAs are involved in many important biological processes, such as cell proliferation, differentiation, and apoptosis.
- d. Recent studies estimate that more than one-third of the cellular transcriptome is regulated by miRNAs.

12. Circulating miRNAs are intensely evaluated as promising blood-based biomarkers. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:827-834.]

- a. Derelegation of miRNA expression levels has been detected in many human tumor types and plays a critical role in cancer pathogenesis.
- b. In blood, miRNAs can circulate withstanding degradation through their inclusion in microvesicles or exosomes that are secreted from cells or by binding to high-density lipoproteins or to the argonaute 2 protein complex.
- c. Endogenous circulating miRNA levels are unstable when plasma is stored at 4°C; therefore, samples should be stored at -20°C, where the extracted miRNAs remain stable for up to two years.
- d. A technical hurdle to the study of miRNA expression is the ability to reliably and efficiently extract miRNAs from biological samples because of their small size and their attachment to lipids and proteins.