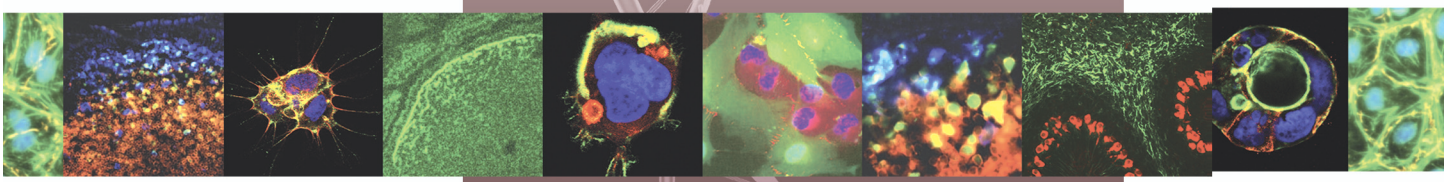
ASIP Journal CME Program

Answer Booklet for the *JMD* 2013 CME Program in Molecular Diagnostics

2013 CME Exam Questions



For more information on how to participate in the 2014 ASIP Journal CME Program see inside or visit:

www.asip.org/CME/

Program Director: Mark E. Sobel, MD, PhD

American Society for Investigative Pathology
Investigating the Pathogenesis of Disease

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JMD CME PROGRAM IN MOLECULAR DIAGNOSTICS

Dear Colleague,

The JMD 2013 CME Program in Molecular Diagnostics was organized as an annual program in which participants were awarded CME credit by successfully answering questions on selected articles in each bimonthly issue of the Journal. The JMD 2013 CME Program in Molecular Diagnostics offered 8 *AMA PRA Category 1 Credit(s)*™ for the successful completion of each bimonthly exam. The American Board of Pathology approved this program for SAM credits (maximum of 48 credits for the year).

We are pleased to provide the Answer Booklet for the six 2013 CME exams on the following pages.

The JMD 2014 CME Program in Molecular Diagnostics is organized in the same way. For your convenience the official CME Accreditation Statement for the 2014 program with subscription information is included at the back of this booklet. Please visit the Journal CME website http://www.asip.org/CME/JMD_2014.cfm for more information. **Members of the Association for Molecular Pathology** (**AMP) and ASIP members receive a 40% discount on the subscription fee for the annual program and may receive a maximum of 48 *AMA PRA Category 1 Credit(s)*™ if all 6 bimonthly exams are successfully completed.** You can achieve credit for each exam successfully completed; it is not necessary to complete all 6 exams.

Special note to AMP members: Due to the transition of a new membership database system for AMP, AMP members were not offered subscription to the JMD 2014 CME Program in Molecular Diagnostics when they renewed their membership. To take advantage of the membership discount, please complete the subscription form at the back of this booklet and submit it to the ASIP Education Office or download it from the JMD CME website at http://www.asip.org/CME/JMD_2014.cfm.

I gratefully acknowledge the American Society for Clinical Pathology for sponsoring the ACCME-accredited JMD CME Program in Molecular Diagnostics since July 2012.

Sincerely yours,

Mark E. Sobel, MD, PhD
Executive Officer
Director of ASIP Journal CME Programs

CONTINUING MEDICAL EDUCATION (CME) INFORMATION

CME Accreditation Statement: This activity ("2013 *JMD* CME Program in Molecular Diagnostics") has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the American Society of Clinical Pathology (ASCP) and the American Society for Investigative Pathology (ASIP). ASCP is accredited by the ACCME to provide continuing medical education for physicians.

The ASCP designates this journal-based CME activity ("2013 *JMD* CME Program in Molecular Diagnostics") for a maximum of 48 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

The 2013 *JMD* CME Program in Molecular Diagnostics is an annual program consisting of a series of 48 questions based on selected articles in the 2013 issues (Volume 15) of *The Journal of Molecular Diagnostics* (*JMD*). Bimonthly exams, consisting of 8 questions that are based on selected articles appearing in each issue of the Journal, will be available online on the Journal website for registered participants.

To receive CME credit for this journal-based CME activity, participants must achieve a score of at least 75% on each bimonthly exam and complete a Post-Test Evaluation. All exams must be completed by December 31, 2013 to receive CME credit. Participants will earn 8 *AMA PRA Category 1 Credit(s)*™ for the successful completion of each bimonthly exam (a minimum of 6 questions answered correctly for each bimonthly exam).

For more information regarding the CME Disclosure Policy and educational objectives, or to subscribe to the 2013 *JMD* CME Program in Molecular Diagnostics, please contact the ASIP Education Office by phone at (301) 634-7440; email (journalcme@asip.org), or mail your inquiry to 9650 Rockville Pike, Suite E-133, Bethesda, MD 20814.

SAM Credit

The 2013 *JMD* CME Program in Molecular Diagnostics is approved by the American Board of Pathology for up to 50 SAM credits. Physicians should only claim credit commensurate with the extent of their participation in the activity. After successfully completing the bimonthly CME exams as described above, participants may separately apply for SAM credit by completing the SAM application found on the ASIP website (http://www.asip.org/CME/documents/ASIP2013JMDSAMApplication.pdf). All SAM applications must be received in our office by December 31, 2013 for participants to receive SAM credit.

For more information regarding SAM credits, please contact the ASIP Education Office by phone at (301) 634-7440; email (journalcme@asip.org), or mail your inquiry to 9650 Rockville Pike, Suite E-133, Bethesda, MD 20814.

Meeting Objective/Target Audience

The objective of the 2013 *JMD* CME Program in Molecular Diagnostics is to increase basic and applied pathology knowledge, focusing on the molecular pathogenesis, diagnosis, prognosis, and the treatment of disease. The 2013 *JMD* CME Program in Molecular Diagnostics is designed to meet the participants' education needs in the physician competency area of Medical Knowledge, as

defined by the Accreditation Council for Graduate Medical Education (ACGME) and the American Board of Medical Specialties (ABMS), and to support participants' lifelong learning towards a goal of promoting patient safety and improving patient care and is specifically targeted to trainees, clinicians and researchers investigating mechanisms of disease who wish to advance their knowledge of the cellular and molecular biology of disease.

Educational Objectives
At the completion of the 2013 *JMD* CME Program in Molecular Diagnostics, participants should be able to:

1. discuss the research underway and/or current molecular approaches to the diagnosis and prognosis of inherited diseases and syndromes;
2. discuss the research underway and/or current molecular approaches to pharmacogenetics, cytogenetics, DNA identity tests, and hematopathology (including clonality, translocations, and point mutations);
3. discuss the research underway and/or current molecular approaches to the diagnosis and prognosis of solid and soft tissue tumors;
4. discuss the research underway and/or current molecular approaches to the diagnosis of infectious diseases (including bacterial, fungal, viral, and parasitic pathogens);
5. discuss the research underway and/or current molecular approaches to the diagnosis and prognosis of acquired diseases spanning systems biology;
6. demonstrate a gained level of knowledge of the molecular methods and techniques being used by researchers and practitioners;

Disclosure of Financial Relationships and Resolution of Conflicts of Interest
In order to ensure balance, independence, objectivity and scientific rigor in all its educational activities, and in accordance with ACCME Standards, the ASCP requires that all individuals in a position to influence and/or control the content of ASCP CME activities disclose to the ASCP and subsequently to learners whether they do or do not have any relevant financial relationships with proprietary entities producing health care goods or services that are discussed in CME activities.

Faculty are asked to use generic names in any discussion of therapeutic options, to base patient care recommendations on scientific evidence and to base information regarding commercial products/services on scientific methods generally accepted by the medical community. All ASCP CME activities are evaluated by participants for the presence of any commercial bias and thus input is used for subsequent CME planning decisions. The primary purpose of this journal-based CME activity is educational and the comments, opinions, and/or recommendations expressed by the faculty or authors are their own and not those of ASCP or ASIP.

The planning committee members and staff of this journal-based CME activity have no relevant financial relationships with commercial interest to disclose. Relevant financial relationships of the authors of selected articles in this journal-based CME activity will be disclosed in a footnote to the published article and in each examination.

ASIP 2013 Journal CME Programs

JMD 2013 CME Program in Molecular Diagnostics

American Society for Investigative Pathology *and the* Association for Molecular Pathology

The Journal of Molecular Diagnostics, Volume 15, Number 1 (January 2013)

www.asip.org/CME/journalCME.htm

Mark E. Sobel, MD, PhD, Director of Journal CME Programs

ANSWERS for CME January Questions # 1-12
1a, 2c, 3d, 4c, 5b, 6d, 7c, 8d, 9a, 10b, 11d, 12d

A Review on DNA methylation profiling, a research article with related Commentary on acute myeloid leukemia (AML), and a research article concerning myotonic dystrophy type I (DM1) were selected for the **January 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 #1-6 are based on: Shanmuganathan R, Basheer NB, Amirthalingam L, Muthukumar H, Kaliaperumal R, Shanmugam K: Conventional and noanotechniques for DNA methylation profiling. J Mol Diagn 2013, 15:17-26; http://dx.doi.org/10.1016/j.jmoldx/2012.06.007.

Questions #7-10 are based on: Spencer DH, Abel HJ, Lockwood CM, Payton JE, Szankasi P, Kelley TW, Kulkarni S, Pfeifer JD, Duncavage EJ: Detection of *FLT3* internal tandem duplication in targeted, short-read-length, next-generation sequencing data. J Mol Diagn 2013, 15:81-93; http://dx.doi.org/10.1016/j.jmoldx/2012.08.001 and the related Commentary by Wertheim GBW, Daber R, Bagg A: Molecular diagnostics of acute myeloid leukemia: it's a (next) generational thing. Am J Pathol 2013, 182:21-28; J Mol Diagn 2013, 15:17-26; http://dx.doi.org/10.1016/j.jmoldx/2012.08.002.

Questions #11-12 are based on: Orpana AK, Ho TH, Alagrund K, Ridanpää, Aittomäki K, Stenman J: Novel heat pulse extension-PCR-based method for detection of large CTG-repeat expansions in myotonic dystrophy type I. J Mol Diagn 2013, 15:110-115; http://dx.doi.org/10.1016/j.jmoldx/2012.07.004.

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

- Understand the biochemistry of DNA methylation and the role it plays in transcriptional regulation.
- Describe the diagnostic implications of DNA-methylation of genes.
- Discuss the advantages and disadvantages of several different techniques used to detect gene methylation.
- Describe the incidence of AML.
- Discuss the laboratory techniques that are used to diagnose AML.
- Describe the fms-related tyrosine kinase 3 gene (*FLT3*) and its importance in AML.
- Describe the molecular pathophysiology of DM1.
- Discuss the advantages and disadvantages of Southern blot analysis and amplification methods in the diagnosis and prognosis of DM1.

1. DNA methylation, an epigenetic alteration, plays a key role in transcriptional control. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:17-26.]

a. DNA methylation occurs when a methyl group is added to the fifth carbon of adenine or to the sixth carbon of cytosine.
b. Aberrant cytosine methylation is associated with silencing of tumor suppressor genes.
c. Alterations in the methylation status of DNA are promising candidates for a highly specific and sensitive indicator of cancer diagnosis and prognosis.
d. DNA methylation is crucial for a variety of processes, such as genomic imprinting, X-chromosome inactivation, and suppression of repetitive elements.

Rationale: DNA methylation occurs when a methyl group is added to the fifth carbon of cytosine or to the sixth carbon of adenine, events that are catalyzed by specific DNA methyltransferases.

2. Changes in DNA methylation in cancer include both global hypomethylation and gene-specific hypermethylation. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:17-26.]

a. Genome-wide hypomethylation results in chromosomal instability and increased mutation rates.
b. Promoter hypermethylation suppresses gene transcription either by preventing transcription factors from binding to the gene or by altering chromatin structure.
c. DNA hypomethylation contributes to oncogenesis by point mutation and inactivation of tumor suppressor genes whereas hypermethylation may lead to chromosomal instability and activation of proto-oncogenes.
d. Tumor growth is characterized by genome-wide hypomethylation, accompanied by hypermethylation of tumor suppressor gene promoters caused by increased expression of DNA methyltransferases.

Rationale: DNA hypermethylation contributes to oncogenesis by point mutation and inactivation of tumor suppressor genes whereas hypomethylation may lead to chromosomal instability and activation of proto-oncogenes.

3. CpG methylation analysis is useful in assessing tumor progression, disease classification, diagnosis, and prognosis of various types of human cancer. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:17-26.]

a. In breast cancer diagnosis, CpG abnormality of genes, such as *ESR1*, *CDKN2A*, *PAR*, *MDGI*, *CALCA*, *CDH1*, *LATS1*, and *LATS2*, is well illustrated.
b. CpG methylation is useful in monitoring the progression of cervical cancer.
c. Promoter-specific CpG methylation aberration is relatively higher in *DAPK1*, *CADM1*, and *CDKN2A* genes in invasive cervical cancers than in high-grade squamous intraepithelial lesions.
d. Methylation analysis cannot be used for risk assessment of patients with prolonged viral and bacterial infections.

Rationale: Methylation analysis is useful for risk assessment of patients with prolonged viral and bacterial infections.

4. Blotting is a conventional technique for DNA methylation analysis. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:17-26.]

a. Antibodies raised against 5-methylcytosine are applied to a DNA sample immobilized on a nitrocellulose membrane to identify 5-methylcytosine in DNA.
b. The 5-methylcytosine sequentially reacts with primary antibody and radiolabeled secondary antibody and is then visualized using autoradiography.
c. The complication of partial renaturation of double-stranded DNA blotted on the nitrocellulose membrane can be eliminated by using reversible denaturation reagents.
d. In concentrated DNA samples, the efficiency of detection is diminished due to steric hindrance caused by the two Fab (antigen-binding fragment) arms of the antibody, which can be avoided by using an Fab instead of the whole antibody.

Rationale: Partial renaturation of double-stranded DNA blotted on the nitrocellulose membrane can be eliminated by using irreversible denaturation agents.

5. Conventional sequencing methods use Maxam and Gilbert chemical cleavage reactions, along with amplification procedures, to establish the methylation status of the promoters in tumor-responsive genes.
Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:17-26.]

a. Conventional sequencing methods can detect protein-binding sites on genomic DNA *in vivo*.
b. It is easy to identify 5-methylcytosine residues using conventional Maxam and Gilbert chemical cleavage reactions.
c. Maxam and Gilbert sequencing protocols cannot be used for small or mixed DNA samples.
d. Bisulfite sequencing is widely used for mapping of promoter hypermethylation and epigenotyping.

Rationale: Major issues exist in the identification of 5-methylcytosine residues in conventional chemical cleavage reaction-based sequencing because a methylated cytosine is identified by the absence of bands in all of the lanes of a sequencing gel, and errors may occur because of indeterminate bands or a background cleavage ladder. Genomic sequencing protocols have been augmented to sodium bisulfate-mediated sequencing to circumvent the existing drawbacks of conventional Maxam and Gilbert sequencing protocols.

6. Methylation status can be assessed on DNA fragments cleaved by methylation-sensitive restriction enzymes using Southern blot hybridization techniques or PCR amplification. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:17-26.]

a. Longer fragments that result from the inability of the enzymes to cleave methylated sequences indicate a methylated CpG dinucleotide.
b. Restriction enzyme-based methods are simple, rapid, and extremely sensitive.
c. Restriction enzyme-based methods are limited to specific restriction sites and require a substantial amount of high-quality DNA.
d. Restriction enzyme-based methods cannot be used for genome-wide methylation analysis and marker discovery techniques.

Rationale: Restriction enzyme-based methods are appropriate for genome-wide methylation analysis and marker discovery techniques.

7. In the United States, acute myeloid leukemia (AML) develops in approximately 13,000 individuals annually and accounts for approximately 9,000 cancer-related deaths per year. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:81-93 and J Mol Diagn 2013, 15:27-30.]

a. Epidemiologically AML is considered a single disease, but it is not a uniform clinicopathologic entity.
b. Some patients respond well to chemotherapeutic regimens, others require hematopoietic stem cell transplants, and yet others rapidly die of AML.
c. The survival of patients with AML is independent of age and performance status.
d. Molecular characteristics of tumor cells are the major factors that influence prognosis of AML.

Rationale: The survival of patients with AML depends in part on tumor-extrinsic factors such as age and performance status.

8. The first recognized and best-studied recurrent genetic lesions in AML are large chromosomal anomalies. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:81-93 and J Mol Diagn 2013, 15:27-30.]

a. The large chromosomal abnormalities are usually detected by metaphase cytogenetic analysis.
b. Translocations and inversions such as t(8;21), inv(16), and t(15;17) confer a relatively good prognosis.
c. Chromosomal gains and losses such as monosomy 7 confer a poor prognosis.
d. All AML cases can be recognized by either traditional karyotyping or by fluorescence *in situ* hybridization (FISH).

Rationale: Some AML cases do not display any abnormalities that can be recognized by either traditional karyotyping or by FISH. Cases with normal karyotypes account for approximately 40% to 50% of AML diagnoses.

9. The fms-related tyrosine kinase 3 gene (*FLT3*) encodes a class III receptor tyrosine kinase that is required for normal hematopoiesis. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:81-93 and J Mol Diagn 2013, 15:27-30.]

a. Recurrent mutations in *FLT3* have been found in approximately 50% of AML cases overall.
b. Recurrent *FLT3* mutations fall into two broad categories: internal tandem duplications (ITDs) within the juxtamembrane domain and point mutations within the kinase domain.
c. Both ITDs within the juxtamembrane domain and point mutations within the kinase domain of mutations render the kinase constitutively active.
d. Only the ITD mutation within the juxtamembrane domain has been definitively shown to correlate with prognosis.

Rationale: Recurrent mutations in *FLT3* have been found in approximately 20% of AML cases overall, and in approximately 30% of normal-karyotype AML cases.

10. Recent genomic studies have demonstrated that besides *FLT3* mutations, recurrent mutations in several other genes may be informative prognostic markers in AML. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:81-93 and J Mol Diagn 2013, 15:27-30.]

a. Mutations in *NPM1*, *CEBPA*, *WT1*, *KIT*, *DNMT3A*, *IDH1*, *IDH2*, *TET2*, and *ASXL1* have all been found to occur in a significant fraction of AML patients.
b. Mutations in *RUNX1*, *MLL*, and *NRAS* are not associated with AML.
c. Focal testing of exons and known hotspots for mutations in some genes is currently available.
d. Next-generation sequencing (NGS)-based approaches are theoretically capable of identifying all types of mutations observed in AML.

Rationale: Mutations in *NPM1*, *CEBPA*, *WT1*, *KIT*, *DNMT3A*, *IDH1*, *IDH2*, *TET2*, *ASXL1*, *RUNX1*, *MLL*, and *NRAS* have all been found to occur in a significant fraction of AML patients.

11. Myotonic dystrophy type 1 (DM1) is an autosomal-dominant disease. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013,15:110-115.]

a. DM1 is caused by an expansion of CTG repeats in the 3' untranslated region of the dystrophia myotonica protein kinase gene (*DMPK*).
b. Unaffected individuals carry two alleles of 5 to 34 CTG-repeat units.
c. DM1 patients carry an expanded mutant allele of more than 50 CTG repeats, sometimes more than 2,000 repeat units.
d. The disease severity is independent of the number of CTG repeats.

Rationale: The number of CTG repeats correlates with the disease severity.

12. Southern blot analysis of enzymatically digested genomic DNA is a widely used method for molecular diagnostics of CTG expansions in DM1. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013,15:110-115.]

a. Southern blot analysis is time consuming, low through-put, and requires large amounts of good-quality genomic DNA.
b. PCR-based methods represent a promising strategy for DM1 detection.
c. A detection limit of ~80 to 100 CTG repeats is common in conventional PCR methods used for molecular diagnostics of DM1.
d. Expansions of ~12,000 CTG repeats have been detected after direct visualization of PCR products on agarose gels using various modifications of PCR reagents and enzymes.

Rationale: Expansions of ~800 CTG repeats have been detected after direct visualization of PCR products on agarose gels using various modifications of PCR reagents and enzymes.

ASIP 2013 Journal CME Programs

JMD 2013 CME Program in Molecular Diagnostics

American Society for Investigative Pathology *and the* Association for Molecular Pathology

The Journal of Molecular Diagnostics, Volume 15, Number 2 (March 2013)

www.asip.org/CME/journalCME.htm

Mark E. Sobel, MD, PhD, Director of Journal CME Programs

ANSWERS for CME March Questions # 1-12
1d, 2c, 3d, 4c, 5d, 6b, 7a, 8b, 9d, 10c, 11c, 12a

A Review on inherited cardiomyopathies, a research article on the detection of expanded triplet repeats in Huntington disease (HD) alleles, and a research article describing a novel detection method for the Chikungunya virus (CHIKV) were selected for the **March 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 #1-7 are based on: Teekakirikul P, Kelly MA, Rehm HL, Lakdawala NK, Funke BH: Inherited cardiomyopathies: Molecular genetics and clinical genetic testing in the postgenomic era. J Mol Diagn 2013, 15: 158-170; http://dx.doi.org/10.1016/j.jmoldx.2012.09.002

Questions #8-10 are based on: Chen H, Takei F, Koay ES, Nakatani K, Chu JJ: A novel DANP-coupled hairpin RT-PCR for rapid detection of Chikungunya virus. J Mol Diagn 2013, 15: 227-233; http://dx.doi.org/10.1016/j.jmoldx.2012.10.004

Questions #11-12 are based on: Jama M, Millson A, Miller CE, Lyon E: Triplet repeat primed PCR simplifies testing for Huntington disease. J Mol Diagn 2013, 15: 255-262; http://dx.doi.org/10.1016/j.jmoldx.2012.09.005

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

- Discuss the genetic etiologies of inherited cardiomyopathies including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), left ventricular noncompaction (LVNC), and restrictive cardiomyopathy (RCM).
- Describe diagnostic testing in the postgenomic era of HCM, DCM, ARVC, LVNC, and RCM.
- Describe the Chikungunya (CHIKV) virus and the diagnosis of CHIKV infection.
- Describe the detection of Huntington disease (HD) alleles.

1. Inherited cardiomyopathies are a group of cardiovascular disorders. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:158-170.]

a. Inherited cardiomyopathies are classified based on ventricular morphology and function.
b. Therapeutic options for inherited cardiomyopathies are limited.
c. Inherited cardiomyopathies include hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), left ventricular noncompaction (LVNC), and restrictive cardiomyopathy (RCM).
d. DCM is the most common of the inherited cardiomyopathies.

Rationale: HCM is the most common inherited cardiomyopathy, with an estimated prevalence of 1 in 500 individuals. HCM is the leading cause of sudden nontraumatic death in young adults and competitive athletes in the United States. Idiopathic DCM has an estimated prevalence of 1 in 2,500 individuals.

2. HCM is characterized by left ventricular hypertrophy (LVH) in the absence of an underlying systemic condition or other cardiac disease, such as valvular heart disease or hypertension. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:158-170.]

a. The age at onset of HCM can range from infancy to old age.
b. HCM manifestations usually do not appear before adolescence in carriers of a pathogenic variant.
c. HCM is primarily inherited in an autosomal recessive pattern.
d. HCM is traditionally diagnosed using cardiac imaging modalities such as echocardiography and cardiac magnetic resonance imaging.

Rationale: HCM is primarily inherited in an autosomal dominant pattern, although reduced penetrance and clinical variability are common.

3. DCM is defined by left ventricle (LV) dilatation and systolic dysfunction. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:158-170.]

a. DCM is the most common indication for cardiac transplantation in the United States.
b. The spectrum of DCM clinical manifestations includes heart failure, thromboembolism, and sudden cardiac death (SCD).
c. DCM can be an end-stage presentation of other diseases or environmental exposures such as myocarditis and alcohol abuse.
d. DCM is mainly a disease of the sarcomere.

Rationale: HCM is mainly a disease of the sarcomere. In contrast, myocyte disarray is absent in DCM. Another difference between HCM and DCM is that contractile dysfunction and ventricular remodeling in DCM are typically progressive.

4. DCM shows a considerable degree of locus heterogeneity. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:158-170.]

a. DCM is most commonly inherited in an autosomal dominant pattern.
b. Desmosomal genes, traditionally known to cause ARVC, may also be involved in the etiology of DCM.
c. Electrophysiologic manifestations of conduction disease, which are associated with variants of the titin gene (*TTN*), usually appear with the onset of DCM.
d. *TTN* may contribute up to 25% of familial and 18% of sporadic DCM cases, making it the most common mutated gene in DCM.

Rationale: Variants in the *LMNA* and *SCN5A* genes are typically associated with DCM and conduction disease. The electrophysiologic manifestations usually appear before the onset of DCM. *TTN*, on the other hand, plays a role in the sarcomere.

5. ARVC is defined by myocyte loss. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:158-170.]

a. Fibrofatty infiltration of the myocardium in ARVC is associated with an increased susceptibility to arrhythmias.
b. ARVC accounts for a significant portion of sudden deaths in athletes and young adults.
c. Both the right ventricle and the LV can be affected in ARVC.
d. The prevalence of ARVC is estimated to be 1 in 500 individuals.

Rationale: The prevalence of ARVC is estimated to be 1 in 2,000 to 5,000 individuals, with 30% to 50% of cases being familial.

6. Isolated LVNC is characterized by a heavily trabeculated or spongy appearance of the LV myocardium. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:158-170.]

a. Myocardial compaction arrest during the first trimester of embryonic development is widely believed to be a cause of LVNC.
b. LVNC is most frequently associated with Barth syndrome, an autosomal condition associated with mitochondrial disorders.
c. Patients with LVNC tend to have early-onset disease, with clinical expression varying from asymptomatic to progressively poor cardiac function, ventricular hypertrophy, increased thromboembolic events, and SCD.
d. Approximately 50% of patients with LVNC also have right ventricular involvement.

Rationale: LVNC is frequently associated with mitochondrial disorders. Barth syndrome is an X-linked condition characterized by early onset cardiomyopathy (usually dilated, sometimes LVNC), neutropenia, muscle weakness, and growth delay.

7. RCM is characterized by increased stiffness of the ventricular chambers. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:158-170.]

a. RCM is characterized by increased ventricular wall thickness and decreased systolic function.
b. Most individuals with RCM develop heart failure and succumb to death within a few years.
c. A clinical overlap between RCM and HCM has been reported.
d. Missense variants in the desmin gene (*DES*) have been identified in several families with desmin-related myopathy, which can present with RCM.

Rationale: Although RCM is characterized by increased stiffness of the ventricular chambers, ventricular wall thickness and systolic function are generally within normal limits.

8. The Chikungunya virus (CHIKV) is an arthropod-borne virus. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:227-233.]

a. CHIKV has been responsible for numerous outbreaks of febrile arthralgia since its discovery in the early 1950s.
b. Chikungunya fever has been documented in nearly 12 countries.
c. From 2005 to 2006, a massive CHIKV outbreak occurred in La Réunion, France, with an estimated 266,000 CHIK cases and >250 deaths.
d. In India, it is estimated that >1.5 million people were infected in 2006 alone.

Rationale: Currently, Chikungunya fever has been documented in nearly 40 countries. In addition to sudden onset of fever, the CHIKV produces headache, fatigue, nausea, vomiting, rashes, myalgia, and severe arthralgia.

9. CHIKV belongs to the *Alphavirus* genus of the *Togaviridae* family. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:227-233.]

a. The CHIKV virus possesses a linear, positive-sense, single-stranded RNA genome.
b. The CHIKV genome is approximately 11.8 kb in length.
c. The CHIKV genome encodes four non-structural proteins (nsP1 to nsP4).
d. The CHIKV genome encodes four structural proteins (C, E2, 6K, and E1) with organization as follows: 5'-cap-nsP1-nsP2-nsP3-nsP4-(junction region)-C-E2-6K-E1-poly(A)-3'.

Rationale: The CHIKV genome encodes five structural proteins (C, E3, E2, 6K, and E1) with organization as follows: 5'-cap-nsP1-nsP2-nsP3-nsP4-(junction region)-C-E3-E2-6K-E1-poly(A)-3'.

10. Diagnosis of CHIKV infection is dependent on virus isolation, detection of virus-specific antibodies by enzyme- linked immunosorbent assay (ELISA), or genomic detection by RT-PCR. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:227-233.]

a. Cell culture-based virus isolation is the gold standard for diagnosis of CHKV infection.
b. Cell culture-based virus isolation requires specialized skills available only in some reference laboratories.
c. An RT-PCR assay was developed for the early detection of the CHIKV using 2,7-diamino-1,8-naphthyridine derivate (DANP)-labeled cytosine bulge hairpin primers to amplify the *nsP3* gene of the CHIKV genome, with a detection limit of 0.03 plaque-forming units (PFUs) per reaction of CHIKV.
d. RT-PCR-based molecular assays are being increasingly used for rapid, early diagnosis, particularly during the acute phase of the illness.

Rationale: The authors developed an RT-PCR assay using DANP-labeled cytosine bulge hairpin primers to amplify a region of the *nsP2* gene of the CHIKV genome. The detection limit was 0.01 PFUs per reaction of CHIKV.

11. Huntington disease (HD) is an autosomal dominant neurodegenerative disorder. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013,15:255-262.]

a. Abnormal expansions of the CAG repeat in the Huntingtin (*HTT*) gene on chromosome 4 are associated with HD.
b. Determination of the number of CAG trinucleotide repeats is routinely used in diagnostic and predictive testing of individuals symptomatic or at risk for HD.
c. The polymorphic CCG repeat in a locus adjacent to the CAG repeat region varies between 3 and 6 triplets in length.
d. The polymorphic CCG repeat includes an apparent CCT site that is usually two or rarely three repeats in length.

Rationale: The polymorphic CCG repeat varies between 7 and 12 triplets in length.

12. When a sample appears homozygous for a normal allele, additional HD testing is recommended to exclude the possibility that an expanded allele was not identified by PCR. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013,15:255-262.]

a. The authors described a chimeric PCR process to easily distinguish true homozygous alleles, making Southern blot analysis unnecessary in all cases.
b. Southern blot analysis is expensive, labor intensive, requires high concentrations of DNA, and can delay turnaround time.
c. Chimeric or triplet repeat primed PCR has been described in screening for fragile X premutations, screening for full mutations, and determination of mosaic fragile X samples.
d. Individuals with juvenile-onset HD usually have larger CAG repeat sizes than adult-onset cases.

Rationale: Chimeric or triplet repeat primed PCR is defined as a PCR method that generates different sized amplicons due to multiple annealing sites on the template. The authors described a chimeric PCR process that was able to identify expanded alleles up to >150 CAG repeats. Very large expanded alleles still require Southern blot analysis.

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ANSWERS for CME May Questions # 1-12
1d, 2a, 3d, 4c, 5a, 6a, 7b, 8d, 9d, 10c, 11d, 12c

Research articles on phosphatidylinositol-3-kinase p110-α catalytic subunit (*PIK3CA*) mutation analysis, mutations in the gene encoding the cystic fibrosis transmembrane conductive regulator (*CFTR*), human papillomavirus (HPV) genotyping, and unclassified variants in mismatch-repair (MMR) genes were selected for the **May 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 #1-4 are based on: Ang D, O'Gara R, Schilling A, Beadling C, Warrick A, Troxell ML, Corless CL: Novel method for *PIK3CA* mutation analysis: Locked nucleic acid-PCR sequencing. J Mol Diagn 2013, 15:312-318; http://dx.doi.org/10.1016/j.jmoldx.2012.12.005

Questions #5-7 are based on: Giordano S, Amato F, Elce A, Monti M, Iannone C, Pucci P, Seia M, Angioni A, Zarrilli F, Castaldo G, Tomaiuolo R: Molecular and functional analysis of the large 5' promotor region of *CFTR* gene revealed pathogenic mutations in CF and *CFTR*-related disorders. J Mol Diagn 2013, 15:331-340; http://dx.doi.org/10.1016/j.jmoldx.2013.01.001

Questions #8-10 are based on: Donà MG, Ronchetti L, Giuliani M, Carosi M, Rollo F, Congiu M, Mazza D, Pescarmona E, Vocaturo A, Benevolo M: Performance of the linear array HPV genotyping test on paired cytological and formalin-fixed, paraffin-embedded cervical samples. J Mol Diagn 2013, 15:373-379; http://dx.doi.org/10.1016/j.jmoldx.2013.01.002

Questions #11-12 are based on: Pérez-Cabornero L, Infante M, Velasco E, Lastra E, Miner C, Durán M: Evaluating the effect of unclassified variants identified in MMR genes using phenotypic features, bioinformatics prediction, and RNA assays. J Mol Diagn 2013, 15:380-390; http://dx.doi.org/10.1016/j.jmoldx.2013.02.003

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

- Describe the mutations that are associated with the phosphatidylinositol-3-kinase p110-α catalytic subunit (*PIK3CA*) and the assays used for mutation detection.
- Understand enrichment methods to enhance mutation detection, including locked nucleic acid (LNA)-PCR.
- Discuss cystic fibrosis (CF) and cystic fibrosis transmembrane regulator gene (*CFTR*)-related disorders (*CFTR*-RDs).
- Understand human papillomaviruses (HPVs) and the limitations of various genetic tests when using formalin-fixed, paraffin-embedded (FFPE) cervical samples.
- Describe hereditary non-polyposis colorectal cancer and the role that mismatch-repair (MMR) genes play in Lynch syndrome.

1. The phosphatidylinositol-3-kinases (PI3Ks) are heterodimeric lipid kinases. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:312-318.]

a. PI3Ks are involved in regulation of cellular growth, transformation, adhesion, apoptosis, survival, and motility.
b. *PIK3CA* encodes the PI3K p110-α catalytic subunit.
c. *PIK3CA* is mutated frequently in invasive breast cancer as well as gastric, colon, lung, brain, endometrial, and other carcinomas.
d. There are three mutational hotspots in *PIK3CA*: codons 542 and 547 of exon 9 (kinase domain) and codon 1005 of exon 20 (helical domain).

Rationale: There are three mutational hotspots: codons 542 and 545 of exon 9 (helical domain) and codon 1047 of exon 20 (kinase domain).

2. Studies have shown that *PIK3CA* mutations are associated with the activation of the downstream PI3K/Akt/mTOR signaling. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:312-318.]

a. Point mutations in the three *PIK3CA* mutational hotspot codons account for more than 90% of *PIK3CA* mutations in human cancers.
b. *PIK3CA* mutations are transforming in cell culture.
c. *PIK3CA* mutations are tumorigenic when overexpressed in the mammary gland in mouse models.
d. *PIK3CA* mutations have prognostic and therapeutic implications.

Rationale: Point mutations in the three *PIK3CA* codons account for more than 80% of *PIK3CA* mutations in human cancers.

3. Several assays have been developed to identify *PIK3CA* mutations. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:312-318.]

a. With the ongoing development of pharmacological agents targeting the PI3K pathway, detection of *PIK3CA* mutations will become increasingly important.
b. High-resolution DNA melting analysis (HRM), pyrosequencing, and real-time PCR are among the assays that can identify *PIK3CA* mutations.
c. Direct Sanger sequencing is one of the most commonly applied methods for the detection of *PIK3CA* mutations.
d. Direct Sanger sequencing has a low mutation detection sensitivity of about 5% to 10%.

Rationale: Direct Sanger sequencing has a low mutation detection sensitivity of about 15% to 25%.

4. Locked nucleic acid (LNA)-PCR is an enrichment method that can enhance mutation detection. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:312-318.]

a. LNA is a nucleic acid analog that contains a 2'-O,4'-C-methylene bridge in the ribose moiety.
b. When incorporated into a DNA oligonucleotide, LNA raises its thermal stability with complementary DNA.
c. The authors optimized the LNA-PCR assay to completely block wild-type DNA amplification.
d. Decreasing the concentration of LNA oligonucleotide decreased the resultant mutant:wild-type peak height ratio.

Rationale: When higher concentrations of LNA oligonucleotides are used, wild-type DNA amplification is suppressed, and mutant allele detection is more sensitive. However, this assay strategy requires a concurrent standard Sanger PCR for every sample to control against PCR failures. The authors optimized the concentration of LNA oligonucleotide such that amplification of wild-type alleles was not completely suppressed, yet mutant alleles were preferentially amplified.

5. Patients with cystic fibrosis (CF) manifest a multisystemic disease due to mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (*CFTR*). Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:331-340.]

a. CF is the most frequent lethal inherited disorder among white people, with an incidence of 1:1000 newborns.
b. The diagnosis of CF is based on symptoms, sweat chloride levels, and molecular analysis findings.
c. CF patients manifest alterations of the chloride channel expressed by most epithelial cells.
d. Causative mutations are identified in 90% to 95% of CF chromosomes using scanning procedures to analyze whole

7. The region at the 5' of *CFTR* may have a relevant role in the regulation of *CFTR* expression. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:331-340.]

a. The authors studied the 6000-bp region at the 5' of *CFTR* in a group of 118 unrelated Italian patients affected by CF or *CFTR*-related diseases (RDs) and identified 23 mutations.
b. The c.-3966T>C allele had a relatively low frequency of 2.0% in patients with *CFTR*-RDs and 4.6% in patients with CF.
c. The c.-5671C>T allele had a high frequency ranging from 24.2% in patients with *CFTR*-RDs to 75.9% in patients with CF.
d. Unlike mutations of *CFTR* coding regions (whose effects involve CFTR activity in all cells), the mutations in the promoter region may have a different effect on different tissues, thus influencing the clinical expression of CF in the single patient.

Rationale: The c.-3966T>C allele had a high frequency ranging from 20.0% in patients with *CFTR*-RDs to 71.6% in patients with CF.

8. Human papillomaviruses (HPVs) are the causative agents of both benign and malignant lesions of the uterine cervix. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:373-379.]

a. HPVs are associated with a subset of other ano-genital cancers and head and neck squamous cell carcinoma.
b. HPVs seem to be involved in the development of nonmelanoma skin cancer.
c. A limited number of HPV genotypes have a role in the development of cervical cancer.
d. HPV 16 is present in >90% of all cervical cancers worldwide.

Rationale: Of the more than 100 HPV genotypes that have been identified to date, twelve HPV genotypes have been classified as carcinogenic or high risk. HPV 16 is present in >60% of all cervical cancers worldwide.

9. The carcinogenic potential of the various HPV genotypes varies. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:373-379.]

a. The risk of developing high-grade cervical lesions and cancer depends on the genotype(s) responsible for the infection.
b. Accurate assessment of the spectrum of genotypes present in the uterine cervix is a major step toward reliable evaluation of cancer risk.
c. A method based on amplification of a small fragment (65 bp) within the L1 region of the HPV genome is among the most valid for testing formalin-fixed paraffin-embedded (FFPE) samples.
d. HPV test results are the same whether xylene or high-heat treatment are used to remove paraffin in FFPE samples.

Rationale: In general, FFPE samples do not provide the most appropriate material for PCR-based assays because formalin fixation induces fragmentation of the nucleic acids, thus decreasing the amount of DNA suitable as a template. Because of the fragmentation induced by fixation, success of PCR amplification from FFPE tissue samples increases with the decrease in template size. DNA recovery from FFPE specimens was improved in terms of both quality and quantity if the xylene treatment to remove paraffin was replaced by high-heat treatment. In general, HPV test results are affected by the method used for DNA extraction before HPV detection.

10. HPV genotype test results partially depend on the source and type of specimen . Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 373-379.]

a. In the described study, 10 biopsy samples tested positive for only one HPV genotype, whereas the corresponding cytologic scraping showed multiple infections.
b. For the most part, cervical scrapings provide superficial cells of the uterine cervix, whereas biopsy specimens provide full-thickness tissue samples.
c. Typically, both cervical scrapings and biopsy specimens are taken from several areas of the uterine cervix.
d. Although it is plausible that only one HPV genotype is responsible for high-grade cervical intraepithelial neoplasia (CIN), it is also possible that multiple infections in FFPE samples are not easily detectable because preferential amplification of one HPV genotype, present at a higher viral load, may occur.

Rationale: For the most part, cervical scrapings provide superficial cells from several areas of the uterine cervix, whereas full-thickness biopsy specimens are collected from a specific area.

11. Hereditary non-polyposis colorectal cancer (HNPCC), also known as Lynch syndrome, is the most frequent autosomal dominant colorectal cancer susceptibility syndrome. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:380-390.]

a. Lynch syndrome is characterized by a high risk of early onset of colorectal cancer and several extracolonic malignant tumors, especially endometrial cancer in women.
b. The phenotype of tumors from patients with Lynch syndrome is characterized by widespread microsatellite instability (MSI) and loss of protein expression from the affected enzyme.
c. Most of the genetic defects in the human mismatch repair (MMR) genes responsible for HNPCC are a result of point mutations and small insertions and deletions that truncate and inactivate MMR genes.
d. Mutations in *PMS2* account for the majority of the patients with Lynch syndrome.

Rationale: Mutations in *MLH1*, *MSH2*, and *MSH6* account for the majority of patients with Lynch syndrome.

12. A major difficulty in diagnosis and management of Lynch syndrome is the existence of unclassified genetic variants (UVs) with unknown clinical significance. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:380-390.]

a. UVs are nucleotide substitutions that are missense but generally not truncating.
b. Missense-type mutations occur in 24% of all unique variants detected in *MLH1*.
c. Missense-type mutations occur in 7% of all unique variants in *MSH2*.
d. Rather than causing changes to a single amino acid, many variants are instead associated with defects in RNA splicing.

Rationale: In the MMR genes, missense-type mutations account for the most common type of mutations detected. Missense-type mutations occur in 17% of all unique variants in *MSH2*, as estimated from the Mismatch Repair Genes Variant Database. In addition to occurring in *MLH1* and *MSH2*, missense-type mutations occur in 27% of all unique variants detected in *MSH6*.

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ANSWERS for CME July Questions # 1-12
1a, 2b, 3a, 4c, 5a, 6d, 7c, 8b, 9a, 10d, 11b, 12d

A Technical Advance article on genetic evaluation of stillbirth using formalin-fixed, paraffin-embedded (FFPE) tissue and research articles on the development of a genomic DNA reference material panel for myotonic dystrophy type 1 (DM1) genetic testing and the development of next-generation sequencing assays for mitochondrial and nuclear genes associated with mitochondrial disorders were selected for the **July 2013 JMD CME Program in Molecular Diagnostics**.

Questions #1-6 are based on: Rowe LR, Thaker HM, Opitz JM, Shiffman JD, Haddadin ZM, Erickson LK, Smith ST: Molecular inversion probe array for the genetic evaluation of stillbirth using formalin-fixed, paraffin-embedded tissue. J Mol Diagn 2013, 15:466-472; http://dx.doi.org/10.1016/j.jmoldx.2013.03.006. The planning committee members and staff have no relevant financial relationships with commercial interests to disclose. The authors have no relevant financial relationship to disclose except for Sarah T. Smith and Joshua D. Schiffman, who received honoraria from Affymetrix for speaking engagements, and Sarah T. Smith, who serves on Affymetrix's Oncology Advisory Board. These relationships were deemed not to be relevant to the educational activity.

Questions #7-10 are based on: Kalman L, Tarleton J, Hitch M, Hegde M, Hjelm N, Berry-Kravis E, Zhou L, Hilbert JE, Luebbe EA, Moxley III RT, Toji L: Development of a genomic DNA reference material panel for myotonic dystrophy type 1 (DM1) genetic testing. J Mol Diagn 2013, 15:518-525; http://dx.doi.org/10.1016/j.jmoldx.2013.03.008. The planning committee members and staff have no relevant financial relationships with commercial interests to disclose. The authors have no relevant financial relationship to disclose except for Madhuri Hegde, who received honoraria as scientific advisor to Genome Quest, RainDance, Tessarar, and Oxford Genetic Technologies. These relationships were deemed not to be relevant to the educational activity.

Questions #11-12 are based on: Dames S, Chou L-S, Xiao Y, Wayman T, Stocks J, Singleton M, Eilbeck K, Mao R: The development of next-generation sequencing assays for the mitochondrial genome and 108 nuclear genes associated with mitochondrial disorders. J Mol Diagn 2013, 15:526-534; http://dx.doi.org/10.1016/j.jmoldx.2013.03.005. The authors of the referenced articles and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

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

- Describe the evaluation of genomic alterations in formalin-fixed, paraffin-embedded (FFPE) stillbirth autopsy tissue.
- Understand molecular inversion probe (MIP) array analysis.
- Describe myotonic dystrophy type 1 (DM1).
- Discuss genetic tests for DM1.
- Describe a new publicly available human cell line-based genomic DNA reference material panel for DM1 genetic testing.
- Understand mitochondrial disorders.
- Describe the mitochondrial and nuclear genes associated with mitochondrial disorders
- Discuss the PCR enrichment methods that are utilized to detect mitochondrial disorders.

1. Stillbirth is defined as the intrauterine death of a fetus during the late second or third trimester of pregnancy, but before delivery. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:466-472.]

a. Stillbirth is defined as the intrauterine death of a fetus with a gestational age >12 weeks.
b. The number of global stillbirths in 2009 was estimated to be 2.64 million; however, this rate is likely to be underrepresented because of a lack of reporting in developing countries.
c. In the United States, ~26,000 stillbirths occur each year.
d. Fetal loss that is unexplained by fetal, placental, maternal, or obstetric influences represents between 25% and 60% of all fetal deaths.

Rationale: Stillbirth is defined as the intrauterine death of a fetus with a gestational age >20 weeks (during the late second or third trimester of pregnancy) before delivery.

2. Determining the cause of stillbirth is essential for effective patient management, counseling, and determination of recurrence risk. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 466-472.]

a. Chromosomal analysis using G-banded karyotype has been considered the gold standard for confirming copy number alterations and structural rearrangements in pregnancy loss.
b. The level of resolution of G-banded karyotyping is 1 to 5 Mb.
c. Traditional cytogenetic analysis cannot be completed in up to 45% to 60% of stillbirth cases owing to culture failure.
d. Selective growth of maternal decidua may lead to the cytogenetic diagnosis of a normal female karyotype that is not representative of the true fetal chromosome complement.

Rationale: The level of resolution of G-banded karyotyping is 10 to 15 Mb, which is inadequate for detecting submicroscopic duplications and deletions that may play a direct role in the etiology of the pregnancy loss.

3. Postmortem autopsy is a useful means for helping to determine the cause of fetal death. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 466-472.]

a. The results of fetal autopsy have been reported to change the clinical diagnosis of the cause of fetal death or to yield additional findings in 12% to 26% of cases.
b. Formalin-fixed, paraffin-embedded (FFPE) tissues processed from fetal autopsy samples offer an alternative to cytogenetic analysis, sidestepping the challenges associated with short-term culture of fetal cells.
c. An advantage of using FFPE tissues is that analysis can be performed when viable, fresh tissue no longer remains.
d. FFPE tissues processed from fetal autopsy samples represent an archive of pathologically informative, disease-specific material for genomic profiling.

Rationale: The results of fetal autopsy have been reported to change the clinical diagnosis of the cause of fetal death or to yield additional findings in 22% to 76% of cases.

4. Several obstacles exist when using archival FFPE material for genetic analysis. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 466-472.]

a. Chemical fixation using formalin causes severe cellular degradation and typically results in reduced nucleic acid quality and quantity.
b. Chemical fixation using formalin typically results in a higher rate of PCR failure and a lower pass rate on array comparative genomic hybridization (aCGH).
c. Protein-protein and protein-nucleic acid cross linking that occurs as a result of formaldehyde fixation causes DNA and RNA to be fragmented to an average length of 400 bp.
d. FFPE tissue age contributes to poor-quality DNA.

Rationale: Protein-protein and protein-nucleic acid cross linking that occurs as a result of formaldehyde fixation causes DNA and RNA to be fragmented to an average length of 200 bp or less.

5. Molecular inversion probe (MIP) technology is an option for providing high-quality copy number data in FFPE tissue samples. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 466-472.]

a. The MIP is a nucleic acid probe with an approximately 60-bp footprint.
b. The 5' and 3' ends of the MIP are complementary to the genomic target of interest.
c. The MIP's internal region contains a unique barcode tag sequence that identifies the probe, the targeted genomic region, two universal PCR primer sites that are common to all MIPs, and two cleavage sites.
d. MIPs are designed to have a gap delimited by the hybridized ends of the probes; the gap remains over the target site (the SNP of interest).

Rationale: The MIP is a nucleic acid probe with an approximately 40-bp footprint.

6. MIP array analysis of FFPE stillbirth material allows for retrospective evaluation and can be an effective tool for patient management. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 466-472.]

a. In the referenced study, one case contained a 15.84 Mb terminal deletion encompassing the *ZIC2* gene, which was the second consecutive fetal loss for the mother.
b. In the referenced study, one case had a 1.8 Mb loss of 17q12 involving the hepatocyte nuclear factor 1B (*HNF1B*) gene in a fetus noted to have bilateral multicystic dysplastic kidneys.
c. In the referenced study, four sections (20 μm thick) were taken from archived FFPE tissue blocks.
d. In the referenced study, microdissection of the FFPE specimens was performed.

Rationale: No microdissection of the FFPE specimens was performed in the study.

7. Myotonic dystrophy type 1 (DM1) is the most common form of adult muscular dystrophy. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:518-525.]

a. DM1 is a dominantly inherited, multisystem disorder that typically affects skeletal, smooth, and cardiac muscle, the eyes, the brain, and endocrine function.
b. DM1 is also referred to as Steinert disease.
c. Penetrance of DM1 is approximately 50% by age 50 years.
d. There is variable expressivity, and mild cases may be misdiagnosed or undiagnosed.

Rationale: Penetrance of DM1 is approximately 100% by age 50 years.

8. DM1 results from an unstable CTG triplet expansion in the *DMPK* gene. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:518-525.]

a. *DMPK* encodes a serine-threonine kinase.
b. The CTG triplet expansion is in the 5' untranslated region of *DMPK*.
c. *DMPK* is located on chromosome 19q13.3.
d. Individuals not affected by DM1 have 5 to 34 CTG triplet repeats in leukocyte DNA.

Rationale: The CTG triplet expansion is in the 3' untranslated region of *DMPK*.

9. DM1 patients with larger CTG repeats tend to have a more severe phenotype. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:518-525.]

a. The children of patients with *DMPK* alleles in leukocyte DNA with 35 to 49 CTG repeats are asymptomatic.
b. *DMPK* alleles with CTG repeat expansions >49 lead to a wide spectrum of symptoms that characterize the DM1 phenotype.
c. *DMPK* alleles >49 CTG repeats are unstable and may expand in length during meiosis, causing offspring to inherit CTG repeats that are longer than those in the parent.
d. Most DM1 patients display somatic tissue mosaicism in skeletal muscle, heart, and brain, which complicates prediction of the phenotype severity.

Rationale: Patients with *DMPK* alleles in leukocyte DNA with 35 to 49 CTG repeats do not have symptoms. However, their children have an increased risk of inheriting larger CTG repeats and of having symptoms.

10. Clinical laboratories use characterized reference materials for quality assurance purposes. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:518-525.]

a. The authors developed a genomic DNA reference material panel for DM1 genetic testing using DM1 cell lines.
b. The three clinical genetics laboratories that participated in the study offer testing for DM1, are located in the United States, and are accredited by the College of American Pathologists.
c. In addition to using existing DM1 cell lines in the NIGMS repository at the Corriell Institute for Medical Research, cell lines were created by Epstein-Barr virus transformation of B lymphocytes from whole blood samples of consenting patients or their families.
d. *DMPK* alleles in the samples characterized for the reference panel cover all five DM1 clinical categories.

Rationale: *DMPK* alleles in the samples cover four of the five DM1 clinical categories: normal (5 to 34 repeats), mild (50 to 100 repeats), classical (101 to 1000 repeats), and congenital (>1000 repeats). No cell lines were identified or established in the premutation range (35 to 49 repeats).

11. Mitochondrial disorders genetically fall into two classes: mutations in the mitochondrial genome (mtDNA) and genes in the human nuclear genome. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:526-534.]

a. mtDNA is a maternally inherited, circular, 16,569-bp haploid organelle composed of 37 genes.
b. It is estimated that up to 400 nuclear genes may be associated with nuclear encoded mitochondrial proteins.
c. Inheritance of nuclear encoded mitochondrial proteins may be autosomal recessive, dominant, or sex-linked.
d. Mitochondrial disorders have an overall incidence of 1:5000.

Rationale: It is estimated that up to 1,500 nuclear genes may be associated with nuclear encoded mitochondrial proteins. Approximately 80% of mitochondrial disease-causing variants are found in the nuclear genome.

12. Because mitochondrial disorders encompass a wide range of phenotypes and a large number of genes, high-throughput next-generation sequencing (NGS) is an ideal method for variant detection. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:526-534.]

a. NGS allows for a low-cost, comprehensive mitochondrial disorder panel.
b. NGS offers the ability to sequence at high coverage, allowing for detection of low-level heteroplasmy for mtDNA mutations, which are not easily detected by Sanger sequencing.
c. Enrichment techniques include long-range PCR (LR-PCR) for the mitochondrial genome and various in-solution and chip-based capture methods for nuclear genes.
d. The authors effectively used blood samples and other tissue types for the mitochondrial disorder assay.

Rationale: A limitation of the current mitochondrial disorder assay is that only blood samples have been used.

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ANSWERS for CME September Questions # 1-12
1b, 2a, 3d, 4d, 5c, 6a, 7c, 8b, 9b, 10c, 11a, 12d

A Special Article on the role of *MGMT* testing in gliomas and research articles on a multiplex qPCR gene dosage assay for large-scale population screening for deletional α-thalassemia, a real-time PCR assay for fusion genes in acute myeloid leukemia (AML), and miRNA profiling in bladder cancer diagnosis were selected for the **September 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 #1-7 are based on: Cankovic M, Nikiforova MN, Snuderl M, Adesina AM, Lindeman N, Wen PY, Lee EQ: The role of *MGMT* testing in clinical practice: A report of the Association for Molecular Pathology. J Mol Diagn 2013, 15:539-555; http://dx.doi.org/10.1016/j.jmoldx.2013.05.011.

Questions #8-9 are based on: Zhou W, Wang G, Zhao X, Xiong F, Zhou S, Peng J, Cheng Y, Xu S, Xu X: A multiplex qPCR gene dosage assay for rapid genotyping and large-scale population screening for deletional α-thalassemia. J Mol Diagn 2013, 15:642-651; http://dx.doi.org/10.1016/j.jmoldx.2013.05.007.

Question #10 is based on: Dolz S, Barragán E, Fuster Ó, Llop M, Cervera J, Such E, De Juan I, Palanca S, Murria R, Bolufer P, Luna I, Gómez I, López M, Ibáñez M, Sanz MA: Novel real-time polymerase chain reaction assay for simultaneous detection of recurrent fusion genes in acute myeloid leukemia. J Mol Diagn 2013, 15:678-686; http://dx.doi.org/10.1016/j.jmoldx.2013.04.003.

Questions #11-12 are based on: Ratert N, Meyer H-A, Jung M, Lioudmer P, Mollenkopf H-J, Wagner I, Miller K, Kilic E, Erbersdobler A, Wikert S, Jung K: miRNA profiling identifies candidate miRNAs for bladder cancer diagnosis and clinical outcome. J Mol Diagn 2013, 15:695-705; http://dx.doi.org/10.1016/j.jmoldx.2013.05.008.

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

- Discuss the World Health Organization (WHO) classification of gliomas.
- Understand the stratification of low-risk and high-risk groups.
- Describe treatment options for gliomas.
- Understand the molecular mechanism of action of temozolomide for adjuvant therapy for glioma patients.
- Describe the structure of the *MGMT* gene and its promoter.
- Discuss the use of bisulfite treatment of DNA in *MGMT* testing.
- Describe the α-globin gene cluster.
- Understand the spectrum of deletional α-thalassemia.
- Describe recurrent fusion genes in acute myeloid leukemia (AML) and detection of AML molecular rearrangements.
- Describe nonmuscle-invasive and muscle-invasive bladder cancers.
- Understand miRNAs and their potential as biomarkers.
- Describe the expression profiles of miRNAs in bladder cancer.

1. Gliomas encompass a molecularly heterogeneous group of primary brain tumors arising from glial cells. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:539-555.]

a. Astrocytomas and oligodendrogliomas are common subtypes of gliomas.
b. World Health Organization (WHO) grade I gliomas are commonly seen in adults with a peak incidence at 50 years.
c. Approximately 2000 to 3000 low-grade gliomas (LGGs) are diagnosed in the United States annually.
d. The peak incidence of LGGs is between the ages of 35 and 44 years.

Rationale: WHO grade I tumors are rarely seen in adults. In adult populations, LGG generally refers to WHO grade II gliomas, such as diffuse astrocytomas, oligodendrogliomas, and oligoastrocytomas, with a peak incidence between the ages of 35 and 44 years.

2. Although LGGs are slow growing, they are invasive and are associated with considerable morbidity and mortality. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 539-555.]

a. Headache is the most common presenting symptom of LGG.
b. Magnetic resonance imaging is the imaging modality of choice for all glioma subtypes.
c. One scoring system separates LGG into low-risk and high-risk groups according to five risk factors: age, tumor diameter, tumor crossing the midline, histology, and neurological deficits.
d. Even though some LGG patients may enjoy relatively long survival times, almost all cases of LGG eventually progress to a higher grade.

Rationale: Seizure is the most common presenting symptom of LGG. Patients may present with a variety of other symptoms, including headaches and focal neurological deficits.

3. High-grade gliomas are the most common malignant primary brain tumors in adults in the United States. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 539-555.]

a. High-grade gliomas are classified by WHO as grades III and IV.
b. The incidence rate of glioblastoma (GBM) in the United States is 3.2 per 100,000 person-years.
c. GBMs account for approximately 60% to 70% of high-grade gliomas.
d. The median survival of patients with grade IV GBM is 3 years.

Rationale: The median survival is only 12 to 18 months for WHO grade IV gliomas and 2 to 5 years for WHO grade III gliomas. GBMs may present *de novo* (primary GBMs) or may arise from a lower-grade glioma (secondary GBMs).

4. Treatments and outcomes for patients with gliomas vary according to grade. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 539-555.]

a. Treatment options for astrocytomas and oligodendrogliomas include surgery, radiation therapy, and/or chemotherapy.
b. A multicenter, randomized, phase III clinical trial conducted by the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) established radiation therapy with concomitant and adjuvant temozolomide (TMZ) as standard therapy for newly diagnosed GBM.
c. TMZ is an oral chemotherapeutic drug whose antitumor effect is primarily due to alkylation at the O6 and N7 positions of guanine, resulting in inhibition of DNA replication.
d. For recurrent GBM, cetuximab received approval by the U.S. Federal Drug Administration (FDA) in 2007.

Rationale: Cetuximab is an anti-epidermal growth factor rector (EGFR) monoclonal antibody. It was approved by the FDA in 2009 for treatment of KRAS wild type colorectal cancer. For recurrent GBM, bevacizumab received accelerated approved by the FDA in 2009. Bevacizumab is a humanized monoclonal antibody against vascular endothelial growth factor (VEGF) that interferes with the growth and maintenance of tumor blood vessels. Studies of bevacizumab in recurrent GBM have demonstrated improved response rates and 6-month progression-free survival, compared with historical controls.

5. In recent years, O-6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation has been established as a biomarker in patients diagnosed with gliomas. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 539-555.]

a. The MGMT protein is encoded by a single gene located on chromosome band 10q26.
b. *MGMT* is a large gene of >150 kb.
c. *MGMT* contains five coding exons.
d. The *MGMT* gene has a TATA-less, CAT-less promoter containing a CpG island.

Rationale: The *MGMT* gene contains five exons, the first of which is noncoding.

6. Expression of the *MGMT* gene is regulated by methylation-dependent epigenetic silencing. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 539-555.]

a. CpG islands are long (typically >3000 bp) stretches of CG-rich DNA, found primarily in promoter regions.
b. Methylation of cytosines in CpG dinucleotides is a covalent modification catalyzed by DNA methyltransferases.
c. Methylation of a promoter acts to silence transcription of the associated gene by binding to specific methylated DNA-binding proteins that form multiprotein complexes, causing condensation of chromatin and inability to bind RNA polymerase and transcriptonal machinery.
d. Most CpG islands are associated with constitutively active genes and are normally unmethylated.

Rationale: CpG islands are short (typically 300 to 3000 bp) stretches of CG-rich DNA.

7. Most of the *MGMT* molecular assays in clinical use are designed to interrogate the first exon and enhancer. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 539-555.]

a. It is technically difficult to evaluate multiple CpGs.
b. Commonly used methods for detection of *MGMT* methylation include methylation-specific PCR, quantitative real-time PCR, and methylation-specific sequencing.
c. All molecular assays for *MGMT* in clinical settings require bisulfite conversion of DNA before analysis.
d. The *MGMT* promoter contains a 777-bp CpG island with 97 CpG sites.

Rationale: Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) does not require bisulfite conversion. Immunohistochemisty for *MGMT* promoter expression also does not involve bisulfite conversion. However, the most commonly used methods for detection of *MGMT* methylation require bisulfite treatment of DNA before analysis. Bisulfite converts unmethylated cytosine into uracil, whereas methylated cytosine (^{m}C) in a CpG island remains unchanged. The bisulfite-modified DNA is then used as a template for PCR. Bisulfite treatment of DNA is technically the most challenging part of methylation detection, because it leads to further DNA fragmentation. Partial conversion can also be problematic, because it leads to false-positive results.

8. The predominant determinants of α-thalassemia are deletions in the human α-globin gene cluster. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:642-651.]

a. α-Thalassemia is characterized by the decrease or absence of α-globin polypeptide chains.
b. In southern China, the prevalence of α-thalassemia carriers is as high as 4% in the Guangxi area and 12% in the Guangdong area.
c. More than 95% of α-thalassemia genetic defects are deletions of variable size that remove one or both α-globin genes or even the entire gene cluster.
d. In addition to deletion defects, point mutations have been described.

Rationale: α-Thalassemia is found throughout the tropical and subtropical regions. In southern China, the prevalence of α-thalassemia carriers is as high as 17.55% in the Guangxi area and 8.53% in the Guangdong area.

9. The clinical severity of α-thalassemia is directly proportional to the number of α-globin genes affected. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 642-651.]

a. Deletions of a single gene (-α/α) mainly produce silent carriers.
b. Deletions of two genes (--/αα or –α/-α) result in hemoglobin H disease.
c. Deletions of four α-globin genes (--/--) result in hemoglobin Bart's hydrops fetalis.
d. The copy number variation mode of the α-globin genes can be used for designing a quantitative PCR assay that is suitable for mass screening of the carrier frequency in the population and for molecular epidemiology studies.

Rationale: Deletions of two genes (--/αα or –α/-α) result in the α-thalassemia trait. Deletions of three genes (--/-α) result in hemoglobin H disease.

10. Detection of molecular changes contributes to a refined diagnosis and prognostic assessment in acute myeloid leukemia (AML) and enables monitoring of minimal residual disease. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:678-686.]

a. The WHO classification of AML includes several entities characterized by recurrent genetic abnormalities.
b. The molecular rearrangements inv(16)(p13.1q22) or t(16;16)(p13.1;q22) involve *CBFB* and *MYH11*.
c. The acute promyelocytic leukemia (APL) variant carrying t(11;17)(q23;q21) is responsive to all-*trans* retinoic acid.
d. The use of single RT-PCR to detect molecular rearrangements in AML is labor-intensive, costly, and time-consuming.

Rationale: The APL variant t(11;17)(q23;q21) (*ZBTB16-RARA*) is unresponsive to all-*trans* retinoic acid.

11. Urinary bladder cancer is a common cancer in the Western world. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:695-705.]

a. Nonmuscle-invasive bladder cancer (NMIBC) accounts for nearly 50% of urinary bladder cases at initial presentation.
b. NMIBCs include stage Ta and carcinoma *in situ* (cancer is confined to the mucosa) and stage T1 (cancer is confined to the submucosa).
c. The standard for the initial diagnosis and prognostic assessment of bladder cancer is cytoscopy and histopathological analysis of biopsy specimens.
d. Current prognosticators such as tumor grade, stage, size, and multifocality do not accurately reflect clinical outcome.

Rationale: Approximately 75% of bladder cancer cases are confined to the mucosa or submucosa at the initial presentation. Approximately 25% of bladder cases already show tumors with muscle invasion at the initial presentation.

12. miRNAs may be useful as new biomarkers to improve the diagnosis and prognosis of different bladder cancer entities. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:695-705.]

a. miRNAs are small nonprotein coding RNAs of 19 to 24 nucleotides.
b. miRNAs are known to regulate gene expression post-transcriptionally by degrading mRNAs or impairing their translation.
c. Members of the mir-8 family determine the epithelial phenotype of cancer cells.
d. miR-130a and miR-130b are part of the same mir family and their expression is correlated in normal and tumor samples, suggesting that conclusions on common expression behaviors can be drawn based solely on mir family affiliation .

Rationale: Even though they are part of the same mir family, correlation analyses revealed no significant association of the miR-130a/miR-130b miRNA pair. This finding provides persuasive evidence that conclusions on common expression behaviors should not be drawn when based solely on family affiliation.

ASIP 2013 Journal CME Programs

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American Society for Investigative Pathology *and the* Association for Molecular Pathology

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Mark E. Sobel, MD, PhD, Director of Journal CME Programs

ANSWERS for CME November Questions # 1-12
1a, 2b, 3d, 4d, 5d, 6c, 7b, 8c, 9a, 10b, 11b, 12c

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 #1-6 are based on: Gong JZ, Cook JR, Greiner TC, Hedvat C, Hill CE, Lim MS, Longtine JA, Sabath D, Wang YL: Laboratory practice guidelines for detecting and reporting *JAK2* and *MLP* mutations in myeloproliferative neoplasm: A report of the Association for Molecular Pathology. J Mol Diagn 2013, 15:733-744; http://dx.doi.org/10.1016/j.jmoldx.2013.07.002

Questions #7-8 are based on: Xu Z, Chen W, Merke DP, McDonnell NB: Comprehensive mutation analysis of the *CYP21A2* gene: An efficient multistep approach to the molecular diagnosis of congenital adrenal hyperplasia. J Mol Diagn 2013, 15:745-753; http://dx.doi.org/10.1016/j.jmoldx.2013.06.001

Questions #9-10 are based on: Bosdet IE, Docking TR, Butterfield YS, Mungall AJ, Zeng T, Coope RJ, Yorida E, Chow K, Bala M, Young SS, Hirst M, Birol I, Moore RA, Jones SJ, Marra MA, Holt R, Karsan A: A clinically validated diagnostic second-generation sequencing assay for detection of hereditary *BRCA1* and *BRCA2* mutations. J Mol Diagn 2013, 15:796-809; http://dx.doi.org/10.1016/j.jmoldx.2013.07.004

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).

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.

Rationale: In 2005, several groups simultaneously described a point mutation in codon 617 of the protein tyrosine kinase gene *JAK2* in *BCR-ABL1* – negative MPN. This mutation was found in the vast majority of patients with PV and in approximately half of patients with ET or PMF.

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.

Rationale: *MPL* mutations may be present in either *JAK2* mutation-positive or mutation-negative cases. Compared to *JAK2* mutations, *MPL* mutations are found less frequently in PMF and ET.

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.

Rationale: In contrast to the V617F mutation, which involves one amino acid codon, exon 12 mutations affect a larger region, spanning codons 533 to 547. The most frequent exon 12 mutation involves an in-frame deletion of six nucleotides at codons 542 and 543 (N542_E543del); it is present in approximately 40% of V617F-negative PV.

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.

Rationale: *MPL* W515L or W515K mutations are present in patients with PMF and ET at a frequency of approximately 5% and 3%, 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.

Rationale: Using standard curves for both the wild-type and mutant forms, it is possible to perform a calculation of the mutant as a percentage of all of the *JAK2* alleles; however, there is no standardization for measuring quantity. A limitation of allele-specific qPCR is that, in many cases, signal is generated in the mutant PCR reaction at high C_T counts even for negative samples. The clinical utility of quantification of *JAK2* V617F has not yet 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.

Rationale: With quantitative assessment, it has been reported that approximately 20% to 30% of PV patients have less than 25% *JAK2* V617F alleles.

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).

Rationale: The incidence of classic CAH worldwide ranges from 1 in 10,000 to 1 in 20,000 births.

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.

Rationale: Approximately 95% of defective *CYP21A2* genes in CAH fall into three categories: i) approximately 65% to 70% are deleterious mutations derived from pseudogene *CYP21A2P* due to small gene conversions; ii) approximately 5% are spontaneous point mutations; and iii) approximately 25% to 30% are large gene rearrangements generated by unequal meiotic crossing-over.

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.

Rationale: Germline mutations in *BRCA1* and *BRCA2* are implicated as causal factors in the majority of inherited breast and ovarian cancers and up to 10% of all breast cancers diagnosed annually.

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.

Rationale: The Sanger method is well suited to the detection of single-base substitutions and small insertions and deletions. Alternative methods such as multiplex ligation-dependent probe amplification are required to detect larger copy-number changes.

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.

Rationale: miRNAs may act as oncogenes or tumor suppressor genes, depending on their target genes. Half of the miRNA genes in humans are located at fragile chromosomal regions that display deletions, amplifications, or translocations. Therefore, aberrant expression of miRNAs occurs frequently.

12. Circulating miRNAs are intensively 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. Deregulation 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.

Rationale: Endogenous circulating miRNA levels are unstable when plasma is stored at 4°C. Samples should be stored at -70°C, where the extracted miRNAs remain stable for up to one year.

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CME Accreditation Statement: This activity ("*JMD* 2014 CME Program in Molecular Diagnostics") has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the American Society for Clinical Pathology (ASCP) and the American Society for Investigative Pathology (ASIP). ASCP is accredited by the ACCME to provide continuing medical education for physicians.

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- demonstrate a gained level of knowledge of the molecular methods and techniques being used by researchers and practitioners.

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The planning committee members and staff of this journal-based CME activity have no relevant financial relationships with commercial interest to disclose. Relevant financial relationships of the authors of selected articles in this journal-based CME activity will be disclosed in a footnote to the published article and in each examination.

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