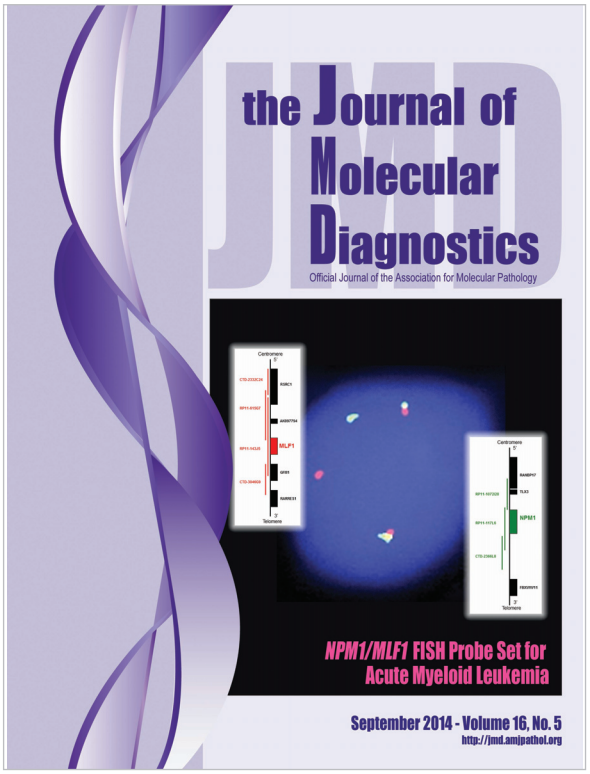
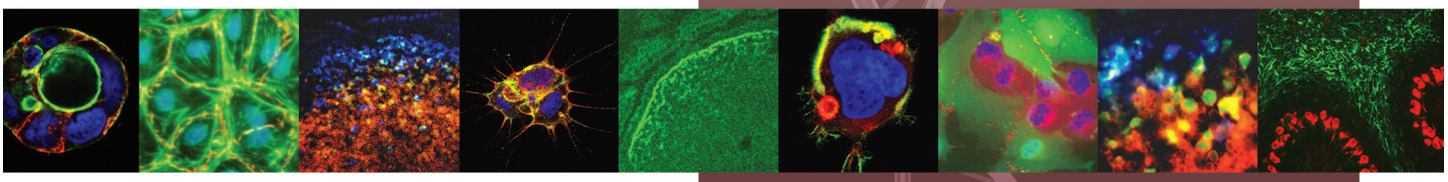


ASIP Journal CME Program

Answer Booklet for the *JMD* 2014 CME Program in Molecular Diagnostics

2014 CME Exam Questions & Answers



For more information on how to participate in the 2015 ASIP Journal CME Program see inside or visit:

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Program Director: Mark E. Sobel, MD, PhD

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Read the articles...take the exams...

Earn CME and Sam Credits!

CME Accreditation Statement
This activity ("*JMD* 2015 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 providership 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.

The ASCP designates this journal-based CME activity ("*JMD* 2015 CME Program in Molecular Diagnostics") for a maximum of 36 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation the activity.

SAM Credit
The *JMD* 2015 CME Program in Molecular Diagnostics is approved by The American Board of Pathology for up to 36 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 available for download on the ASIP website at http://www.asip.org/CME/documents/2015JMD_SAM_App.pdf. All applications must be received in the ASIP office by December 31, 2015 in order for participants to receive SAM credit.

Questions?
ASIP Education Office
9650 Rockville Pike, Bethesda, MD 20814
Tel: 301-634-7440
Email: *journalCME@asip.org*

The ***Journal of Molecular Diagnostics (JMD)* CME Program in Molecular Diagnostics** offers you the opportunity to earn up to 36 CME credits per year while renewing and updating your knowledge in molecular diagnostics. This program is also approved by The American Board of Pathology for up to 36 Self-Assessment Module (SAM) Credits.

The *JMD* 2015 CME Program in Molecular Diagnostics is an annual program consisting of a series of at least 36 questions based on selected articles in the 2015 issues (Volume 17) of *The Journal of Molecular Diagnostics (JMD)*.

To receive CME credit for this journal-based CME activity, participants much 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, 2015 to receive CME credit. Participants will earn 6 *AMA PRA Category 1 Credit(s)*™ for the successful completion of each bimonthly exam.

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American Society for Investigative Pathology
Investigating the Pathogenesis of Disease

MARK E. SOBEL, MD, PHD
EXECUTIVE OFFICER
9650 Rockville Pike, Bethesda, MD 20814-3993 (USA)
Tel: 301-634-7130 • Fax: 301-634-7990 • Email: mesobel@asip.org • www.asip.org

JMD CME PROGRAM IN MOLECULAR DIAGNOSTICS

Dear Colleague,

The JMD 2014 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 2014 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).

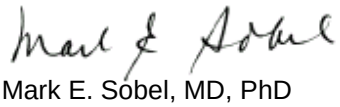
We are pleased to provide the Answer Booklet for the six 2014 CME exams on the following pages.

The JMD 2015 CME Program in Molecular Diagnostics is organized in the same way; however, in 2015 the annual program is accredited for 36 *AMA PRA Category 1 Credit(s)*™ (6 *AMA PRA Category 1 Credit(s)*™ per bimonthly exam) and The American Board of Pathology has approved the program for a maximum of 36 SAM credits for the year. For your convenience the official CME Accreditation Statement for the 2015 program with subscription information is included at the back of this booklet. Please visit the Journal CME website http://www.asip.org/CME/JMD_2015.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 36 *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.

The Journal of Molecular Diagnostics is jointly owned by ASIP and the Association for Molecular Pathology.

I gratefully acknowledge the American Society for Clinical Pathology for joint providership of 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 ("2014 *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 ("2014 *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 2014 *JMD* CME Program in Molecular Diagnostics is an annual program consisting of a series of at least 48 questions based on selected articles in the 2014 issues (Volume 16) of *The Journal of Molecular Diagnostics* (*JMD*). Bimonthly exams, consisting of at least 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, 2014 to receive CME credit. Participants will earn 8 *AMA PRA Category 1 Credit(s)*™ for the successful completion of each bimonthly exam (a score of at least 75% of the questions answered correctly for each bimonthly exam).

For more information 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 2014 *JMD* CME Program in Molecular Diagnostics is approved by the American Board of Pathology for up to 48 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/ASIP2014JMDSAMApplication.pdf). All SAM applications must be received in our office by December 31, 2014 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.

Objective/Target Audience

The objective of the 2014 *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 2014 *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. The program is specifically targeted to pathologists and laboratory professionals who practice molecular pathology and researchers investigating molecular mechanisms of disease, pathology residents and fellows in molecular genetic pathology training programs, and clinicians and researchers interested in advances in molecular diagnostics.

Educational Objectives
At the completion of the 2014 *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 on the published article and in each examination.

ASIP 2014 Journal CME Programs

JMD 2014 CME Program in Molecular Diagnostics

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

The Journal of Molecular Diagnostics, Volume 16, Number 1 (January 2014)

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, 3b, 4d, 5c, 6b, 7a, 8b, 9c, 10a, 11d, 12a

Research articles on high-throughput technology for the diagnosis of genetic cardiomyopathies, the association of the V122I mutation in transthyretin with early-onset cardiac amyloidosis, and age as a criterion for subclassification of early-onset colorectal cancer were selected for the **January 2014 JMD CME Program in Molecular Diagnostics**. The authors of the referenced articles, the planning committee members, and staff have no relevant financial relationships with commercial interests to disclose.

Questions #1-3 are based on: D'Argenio V, Frisso G, Precone V, Boccia A, Fienga A, Pacileo G, Limongelli G, Paolella G, Calabrò R, Salvatore F: DNA sequence capture and next-generation sequencing for the molecular diagnosis of genetic cardiomyopathies. J Mol Diagn 2014, 16:32-44; http://dx.doi.org/10.1016/j.jmoldx.2013.07.008

Questions #4-7 are based on: Reddi HV, Jenkins S, Theis J, Thomas BC, Connors LH, Rhee FV, Highsmith WE: Homozygosity for the V122I mutation in transthyretin is associated with earlier onset of cardiac amyloidosis in the African American population in the seventh decade of life. J Mol Diagn 2014, 16:68-74; http://dx.doi.org/10.1016/j.jmoldx.2013.08.001

Questions #8-12 are based on: Perea J, Rueda D, Canal A, Rodríguez Y, Álvaro E, Osorio I, Alegre C, Rivera B, Martínez J, Benítez J, Urioste M: Age at onset should be a major criterion for subclassification of colorectal cancer. J Mol Diagn 2014, 16:116-126; http://dx.doi.org/10.1016/j.jmoldx.2013.07.010

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

- Discuss the genetic basis of hypertrophic cardiomyopathy (HCM).
- Understand the different approaches for genotyping patients with HCM.
- Describe amyloidosis and its hereditary component.
- Discuss the characteristics and genetic basis of transthyretin amyloidosis (ATTR).
- Define the specific phenotypes associated with transthyretin (*TTR*) mutations.
- Describe early-onset colorectal cancer (CRC).
- Understand the genetic and epigenetic mechanisms that lead to the development of CRC.
- Define the CpG island methylator phenotype (CIMP) in CRC.
- Describe the characteristics and causes of Lynch syndrome.

1. Hypertrophic cardiomyopathy (HCM) is one of the most common inherited cardiac disorders. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:32-34.]

a. The prevalence of HCM in adults is 1 in 700.
b. HCM is a primary myocardial disorder that is inherited in most instances as an autosomal dominant trait.
c. HCM is characterized by hypertrophy of the left ventricle with histologic features of cellular hypertrophy, myofibrillar disarray, and interstitial fibrosis.
d. HCM is the most common cause of sudden cardiac death in young individuals and can contribute to disability at any age.

Rationale: HCM is one of the most common inherited cardiac disorders; its prevalence in adults is 1 in 500.

2. HCM is a disease of remarkable genetic heterogeneity. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:32-34.]

a. Hundreds of HCM mutations have been identified, scattered over more than 30 genes.
b. HCM mutations are essentially found in sarcomeric, ionic-handling channels, and metabolic regulatory proteins.
c. Disease-causing mutations can be found in about 20% to 50% of HCM patients.
d. The phenotypic expression of HCM is believed to result not only from a single disease-causing mutation, but also from mutations in other genes that may act as modifier genes.

Rationale: Disease-causing mutations can be found in about 40% to 60% of HCM patients, suggesting that other disease-causing genes remain to be identified.

3. The genetic heterogeneity, variable penetrance, and the daunting task of analyzing all known candidate genes temper the enthusiasm for genotyping patients with HCM. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:32-34.]

a. Current PCR-based strategies are inadequate for genomic investigations involving many candidate genes.
b. The systematic search for variants in a large number of genes by denaturing high performance liquid chromatography (DHPLC) has been the major approach to genotype HCM.
c. Next-generation sequencing (NGS) generates a large amount of high-quality data.
d. NGS is a new approach to the study of diseases with a heterogeneous genetic basis.

Rationale: Different approaches have been used to address HCM genotyping, namely procedures to test multiple known mutations via array hybridization or the systematic search for variants in a small number of genes by DHPLC or direct Sanger sequencing.

4. Individuals heterozygous for the V122I mutation in transthyretin (*TTR*) tend to develop cardiac amyloidosis, often after the seventh decade of life. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:68-74.]

a. Amyloidosis is characterized by the abnormal deposition of one of a variety of plasma proteins as insoluble β-pleated sheet aggregates, resulting in disruption of organ and tissue function.
b. Hereditary amyloidosis is one of the major subtypes of amyloidosis.
c. Hereditary amyloidosis occurs due to mutations in several genes, including *APOAI*, *APOAII*, *FGA*, *GSN*, *LYZ*, and *TTR*.
d. Mutations in the *TTR* gene account for approximately 70% of hereditary amyloidosis.

Rationale: Mutations in the *TTR* gene are the most common, accounting for approximately 90% of hereditary amyloidosis.

5. Transthyretin amyloidosis (ATTR) is a systemic disease that involves extracellular deposition of amyloid fibrils that accumulate in various organs and tissues. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:68-74.]

a. ATTR can be characterized by sensory, motor, and autonomic neuropathy with or without cardiomyopathy or cardiomyopathy alone.
b. ATTR includes an age-related form known as senile systemic amyloidosis, an acquired disorder that mainly affects men.
c. Senile systemic amyloidosis, which results from the deposition of wild-type ATTR, exhibits an approximately 10 to 30:1 male/female ratio after the age of 65 years.
d. ATTR can be difficult to recognize and manage.

Rationale: Senile systemic amyloidosis is an age-related form of ATTR. An acquired disorder that mainly affects men, senile systemic amyloidosis results from the deposition of wild-type ATTR, primarily in the heart. Senile systemic amyloidosis exhibits an approximately 25 to 50:1 male/female ratio after the age of 75 years.

6. ATTR is an autosomal dominant disorder with >80 known causative mutations. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:68-74.]

a. The *TTR* V122I variant is a common mutation in African Americans, appearing to have originated in West Africa.
b. The heterozygote frequency of the V122I variant is approximately 7% in African Americans.
c. According to a previous study, there should be approximately 13,000 individuals homozygous for the V122I mutation in the United States; however, only 11 homozygotes for the V122I mutation have been reported previously.
d. The current study identified 13 additional homozygous individuals, all of whom were African Americans.

Rationale: The heterozygote frequency of the V122I variant is approximately 4% in African Americans.

7. There is a strong genotype/phenotype correlation in ATTR, with specific *TTR* mutations being associated with purely neurologic disease, purely cardiac disease, or both. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:68-74.]

a. The T60A and V122I variants are associated exclusively with a cardiac phenotype.
b. Heterozygosity for the V122I mutation is associated with cardiac amyloidosis and congestive heart failure.
c. The V122I variant is associated with mortality in African Americans, typically after the age of 70 years.
d. The V30M mutation is primarily associated with neuropathy.

Rationale: The T60A mutation demonstrates both neuropathy and cardiomyopathy. The V122I variant is associated exclusively with a cardiac phenotype.

8. The importance of colorectal cancer (CRC) is undeniable and is underscored by its increasing prevalence in the past years. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:116-126.]

a. Early-onset CRC has an incidence of 2% to 8% of all CRCs.
b. Between 1992 and 2005, CRC incidence increased in the United States at a rate of 3.0% per year in men and 3.5% per year in women.
c. The classic description of colorectal carcinogenesis, the adenoma-carcinoma sequence, has considerably evolved.
d. CRC is increasingly classified into specific phenotypes on the basis of different carcinogenetic pathways.

Rationale: Early-onset CRC has an incidence of 2% to 8% of all CRCs, and between 1992 and 2005 it increased in the United States at a rate of 1.5% per year in men and 1.6% per year in women.

9. An important proportion of early-onset CRC does not show a hereditary component. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:116-126.]

a. Most sporadic CRC cases (60%) show chromosomal instability, with chromosomal translocations and aneuploidy.
b. There are colorectal tumors with microsatellite instability (MSI), whose carcinogenetic pathway is also known as mutator phenotype.
c. The mutator phenotype accounts for 25% of CRCs.
d. In CRCs classified as mutator phenotypes, the *MLH1* DNA mismatch repair (MMR) gene is epigenetically silenced.

Rationale: The mutator phenotype accounts for 15% of CRCs.

10. The analysis of methylation of CpG islands as a mechanism of silencing genes in colon tumors has resulted in the identification of the CpG island methylator phenotype (CIMP). Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:116-126.]

a. CIMP accounts for over 60% of CRCs.
b. CIMP-high tumors have a distinct clinical, pathologic, and molecular profile.
c. CIMP-high tumors are associated with proximal location in the colon, female sex, poor differentiation, MSI, and *BRAF* mutations.
d. Most CIMP-high tumors have an MSI phenotype.

Rationale: CIMP accounts for almost 40% of CRCs.

11. Early onset of cancer is a marker of a potential hereditary component, with Lynch syndrome (LS) being the most frequent form of hereditary CRC. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:116-126.]

a. LS is characterized by earlier development of CRC than sporadic forms.
b. LS is caused by a germline mutation in one of the DNA MMR genes, which leads to MSI.
c. The proportion of MSI tumors found in early-onset CRC varies from 19.7% to 41%, depending on age at onset.
d. LS tumors are mostly found in the left colon.

Rationale: LS tumors are mostly in the right colon. They are frequently poorly differentiated and mucinous tumors, with a tendency to develop synchronous and metachronous CRC.

12. As a whole, there are some controversial aspects about the natural history and prognosis of early-onset CRC. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:116-126.]

a. Studies have identified the *APC* gene as a specific marker for microsatellite-stable (MSS) early-onset CRCs.
b. Early-onset CRC has been shown to be occasionally familial after excluding cases owing to an MMR mutation or adenomatous polyposis.
c. Chromosome 14 may harbor a gene playing an important role in early-onset CRCs that is responsible for an inherited predisposition.
d. Microsatellite- and chromosome-stable tumors seem to be associated with a poor prognosis, an invasive phenotype, and early metastasis.

Rationale: Studies have identified several molecular markers in MSS early-onset CRCs (modified expression of the *APC*, *CTNNB1*, and *TP53* genes), but these molecular features have also been described in sporadic CRC.

ASIP 2014 Journal CME Programs

JMD 2014 CME Program in Molecular Diagnostics

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

The Journal of Molecular Diagnostics, Volume 16, Number 2 (March 2014)

www.asip.org/CME/journalCME.htm

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

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

A Review on the molecular analysis of myelodysplastic syndromes, and research articles on next-generation sequencing of autosomal dominant polycystic kidney disease, the detection of *Clostridium difficile*, and the detection of *Salmonella enterica* species were selected for the **March 2014 JMD CME Program in Molecular Diagnostics**. The authors of the referenced articles, the planning committee members, and staff have no relevant financial relationships with commercial interests to disclose.

Questions #1-6 are based on: Nybakken GE, Bagg A: The genetic basis and expanding role of molecular analysis in the diagnosis, prognosis, and therapeutic design for myelodysplastic syndromes. J Mol Diagn 2014, 16:145-158; http://dx.doi.org/10.1016/j.jmoldx.2013.11.005

Questions #7-8 are based on: Tan AY, Michaeel A, Liu G, Elemento O, Blumenfeld J, Donahue S, Parker T, Levine D, Rennert H: Molecular diagnosis of autosomal dominant polycystic kidney disease using next-generation sequencing. J Mol Diagn 2014, 16:216-228; http://dx.doi.org/10.1016/j.jmoldx.2013.10.005

Questions #9-10 are based on: Angione SL, Sarma AA, Novikov A, Seward L, Fieber JH, Mermel LA, Tripathi A: A novel subtyping assay for detection of *Clostridium difficile* virulence genes. J Mol Diagn 2014, 16:244-252; http://dx.doi.org/10.1016/j.jmoldx.2013.11.006

Questions #11-12 are based on: Masek BJ, Hardick J, Won H, Yang S, Hsieh Y, Rothman RE, Gaydos CA: Sensitive detection and serovar differentiation of typhoidal and nontyphoidal *Salmonella enterica* species using 16S rRNA gene PCR coupled with high-resolution melt analysis. J Mol Diagn 2014, 16:261-266; http://dx.doi.org/10.1016/j.jmoldx.2013.10.011

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

- Discuss myelodysplastic syndromes (MDS) and the affected population.
- Understand the molecular basis of MDS.
- Describe the use of conventional karyotyping and single nucleotide polymorphism arrays in MDS diagnosis..
- Understand the role of single gene molecular alterations in the development of MDS.
- Define MDS DNA methylation status.
- Describe autosomal dominant polycystic kidney disease (ADPKD).
- Understand the genetic basis of ADPKD.
- Describe *Clostridium difficile* and the current methods for its diagnosis.
- Understand *Salmonella enterica* species infections and how they are diagnosed.

1. The myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders of ineffective hematopoiesis. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:145-158.]

a. MDS characteristically demonstrate peripheral blood cytopenia, bone marrow hypercellularity, and morphologically defined dysplasia of one or more hematopoietic lineages.
b. MDS typically affect adults with a median age of 55 years at diagnosis.
c. MDS are recognized as causes of bone marrow failure in the pediatric setting.
d. MDS was once thought of as almost invariably leading to the development of an acute leukemia; however, our understanding of these diseases as a distinct group of disorders has evolved with the recognition that a majority of cases never progress to acute myeloid leukemia (AML).

Rationale: MDS typically affect the elderly (85% diagnosed over the age of 60 years) with a median age at diagnosis of 76 years, although they are also recognized as causes of bone marrow failure in the pediatric setting.

2. The molecular basis for MDS is only beginning to be elucidated, and unifying themes remain elusive, although epigenetic and spliceosome pathways are emerging as frequent targets. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:145-158.]

a. Flow cytometry no longer plays a role in the diagnosis of MDS.
b. Genetic alterations in hematopoietic precursors, likely including stem cells, undoubtedly underlie the distinct natural disease course of MDS subtypes.
c. A diverse group of tests have been developed to test for the molecular changes in MDS, for example, mutations, miRNA, and altered methylation states.
d. Understanding MDS development at a mechanistic level will aid in diagnosis, determination of prognosis, and the generation of novel, directed therapies.

Rationale: Common genetic alterations in MDS occur at a number of different levels, including cytogenetic, submicroscopic, epigenetic, and RNA, requiring multiple testing modalities. Flow cytometry is assuming an increasingly important auxiliary role in diagnosis.

3. Conventional karyotyping, performed on metaphase cells with a Giemsa (G) stain, enables a coarse but very useful genome-wide survey. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:145-158.]

a. The G-banding of 20 metaphase cells is evaluated, revealing most large translocations, gains, and deletions.
b. The karyotype is central to the diagnosis and accurate classification of MDS and essential for prognostication.
c. Karyotypic abnormalities, detected by metaphase chromosomal analysis in approximately 65% of all cases of *de novo* MDS, are less frequent in patients with secondary MDS (~40%).
d. The partial or complete loss of chromosomes followed by partial or complete gains are most characteristic of MDS.

Rationale: Karyotypic abnormalities, detected by metaphase chromosomal analysis in approximately 50% of all cases of *de novo* MDS, are even more frequent in patients with secondary MDS (~80%).

4. Single nucleotide polymorphism (SNP) arrays, with the ability to sensitively detect loss of heterozygosity in tumors, appear to be a useful addition to metaphase cytogenetics by capturing additional cryptic gains or losses. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:145-158.]

a. Use of SNP arrays results in the discovery of more chromosomal abnormalities than by metaphase cytogenetics alone. In patients with normal cytogenetic profiles, the cryptic changes discovered by SNP arrays are often adverse prognostic indicators.
b. In diagnostic MDS specimens, SNP arrays are already starting to become a complementary method for conventional cytogenetics with their own prognostic import.
c. SNP array has been used to distinguish hypocellular MDS from aplastic anemia.
d. SNP analysis is used to detect balanced chromosomal translocations.

Rationale: SNP analysis cannot detect balanced chromosomal translocations and may miss some minor clones.

5. Single gene molecular alterations in MDS are currently being elucidated at a rapid pace. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:145-158.]

a. About 80% of MDS patients have detectable mutations, but no specific mutation is present in more than about 40% of patients with MDS.
b. The vast majority of MDS mutations are low incidence.
c. Cases of secondary MDS are more likely to have mutations than cases of *de novo* MDS.
d. A wide variety of mutations have been identified, including *TET2, RUNX1, TP53, NRAS, ASXL1,* and less commonly *CBL* and *EZH2*.

Rationale: About 70% of MDS patients have detectable mutations, but no specific mutation is present in more than about 20% of patients with MDS.

6. The two most prominent mechanisms in MDS, DNA methylation and histone acetylation and methylation, also play roles in physiologic hematopoiesis. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:145-158.]

a. Seventy percent of high-risk MDS patients had hypermethylation of *ALOX12, GSTM1, HIC1, FZD9,* and *HS3ST2*.
b. MDS is associated on a genomic level with a global increase in methylation.
c. A number of genes that encode proteins that modulate the epigenetic status, including *IDH1/IDH2, TET2,* and *DNMT3A*, are mutated in myeloid malignancies.
d. Although there are strategies for assaying methylation status in other conditions, there is no current standardized clinical assay in MDS.

Rationale: MDS is associated on a genomic level with a global reduction in methylation. This is uneven, however, with focally increased methylation around tumor suppressor genes.

7. Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in *PKD1* and *PKD2*. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:216-228.]

a. ADPKD affects 1 in 50 to 1 in 500 live births worldwide.
b. ADPKD is the most common inherited kidney disease.
c. ADPKD accounts for approximately 5% of the end-stage renal disease population.
d. ADPKD is initiated by gene mutations in renal tubular epithelial cells.

Rationale: ADPKD affects 1 in 400 to 1 in 1,000 live births worldwide.

8. The genetic analysis of ADPKD is complicated by six *PKD1* pseudogenes, large gene sizes, and allelic heterogeneity. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:216-228.]

a. *PKD1* and *PKD2* account for 75% to 85% and 15% to 25% of cases of ADPKD, respectively.
b. *PKD1* spans 46 exons and encodes polycystin-1 with 4,303 amino acids.
c. Chromosome 16 includes six homologous genes that share 97.7% sequence identity with the *PKD1* gene exons 1 to 33.
d. *PKD2* spans 9 exons, encoding polycystin-2, which consists of 1,005 amino acids.

Rationale: *PKD2* spans 15 exons, encoding polycystin-2, which consists of 968 amino acids.

9. *Clostridium difficile* is an anaerobic, spore-forming, Gram-positive bacterium that colonizes the human colon. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:244-252.]

a. *C. difficile* typically presents as an opportunistic infection after other colonic flora have been eradicated by commonly used antibiotics.
b. *C. difficile* infection can cause severe diarrhea, pseudomembranous colitis, and toxic megacolon, and may require urgent colectomy or result in death.
c. *C. difficile* infection is rapidly increasing in incidence, severity, and mortality, particularly in the United States, Canada, and Europe.
d. In the United States, *C. difficile* infection was responsible for $2.5 billion in excess hospital costs in 2008 and an estimated 10,000 deaths from 2006 to 2007.

Rationale: In the United States, *C. difficile* infection was responsible for $4.8 billion in excess hospital costs in 2008 and an estimated 14,000 deaths from 2006 to 2007.

10. The changing epidemiology of *C. difficile* has been marked by hospital outbreaks due to a hypervirulent strain, NAP1/027/BI. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:244-252.]

a. Current methods of diagnosing *C. difficile* include stool culture, toxin testing, enzyme immunoassays, and PCR.
b. Stool culture and cytotoxicity tests provide high sensitivity and specificity.
c. Stool culture and cytotoxicity tests require at least 7 days to complete.
d. Several multiplex PCR assays have been reported to identify various genes associated with *C. difficile*.

Rationale: Although stool culture and cytotoxicity tests provide high sensitivity and specificity, these methods are impractical in most clinical settings because they require 2 to 3 days to complete, during which time clinicians must rely on empirical treatment of disease with antibiotics.

11. *Salmonella enterica* species infections are a significant public health problem. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:261-266.]

a. Each year in the United States, approximately 1 million domestically acquired foodborne illnesses and >350 deaths occur because of nontyphoidal *S. enterica* species infections.
b. Approximately 52.9 million illnesses and 250,000 deaths occur annually worldwide from nontyphoidal *S. enterica* species infections.
c. Typhoidal *S. enterica* species infections cause approximately 21.7 million cases of typhoid fever and >200,000 deaths annually worldwide.
d. Typhoidal and nontyphoidal *S. enterica* species cause high worldwide morbidity rates and high mortality rates in the developing world.

Rationale: Each year approximately 93.8 million illnesses and 155,000 deaths occur worldwide.

12. The global burden of *S. enterica* infections is poorly characterized due, in part, to insufficient diagnostic and surveillance methods and emergence of antimicrobial-resistant *S. enterica* species. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:261-266.]

a. The Centers for Disease Control and Prevention and the World Health Organization have established laboratory-based surveillance programs and guidelines for the detection, identification, treatment, and prevention of *S. enterica* species infections.
b. Not all *S. enterica* species infections are properly diagnosed, leading to delays in adequate treatment and accurate surveillance data.
c. Feces are the only source material from which the diagnosis of an *S. enterica* species infection can be made in a clinical laboratory.
d. 16S PCR coupled with high-resolution melt analysis could be a useful molecular diagnostic method to enhance the current diagnostic, treatment, and surveillance methods of *S. enterica* bloodstream infections.

Rationale: The diagnosis of an *S. enterica* species infection requires isolation of the organism from feces, blood, or other sterile body fluid in a clinical laboratory.

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

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

A Perspective on methods-based proficiency testing in molecular genetic pathology and research articles on the molecular diagnosis of extraskeletal myxoid chondrosarcoma, pathogenicity evaluation of *BRCA1* and *BRCA2* unclassified variants, and automated blood group genotyping were selected for the **May 2014 JMD CME Program in Molecular Diagnostics**. The authors of the referenced articles, the planning committee members, and staff have no relevant financial relationships with commercial interests to disclose.

Questions #1-3 are based on: Schrijver I, Aziz N, Jennings LJ, Richards CS, Voelkerding KV, Weck KE: Methods-based proficiency testing in molecular genetic pathology. J Mol Diagn 2014, 16:283-287; http://dx.doi.org/10.1016/j.jmoldx.2014.02.002

Questions #4-6 are based on: Benini S, Cocchi S, Gamberi G, Magagnoli G, Vogel D, Ghinelli C, Righi A, Picci P, Alberghini M, Gambarotti M: Diagnostic utility of molecular investigation in extraskeletal myxoid chondrosarcoma. J Mol Diagn 2014, 16:314-323; http://dx.doi.org/10.1016/j.jmoldx.2013.12.002

Questions #7-9 are based on: Santos C, Peixoto A, Rocha P, Pinto P, Bizarro S, Pinheiro M, Pinto C, Henrique R, Teixeira MR: Pathogenicity evaluation of *BRCA1* and *BRCA2* unclassified variants identified in Portuguese breast/ovarian cancer families. J Mol Diagn 2014, 16:324-334; http://dx.doi.org/10.1016/j.jmoldx.2014.01.005

Questions #10-12 are based on: Paris S, Rigal D, Barlet V, Verdier M, Coudurier N, Bailly P, Brès JC: Flexible automated platform for blood group genotyping on DNA microarrays. J Mol Diagn 2014, 16:335-342; http://dx.doi.org/10.1016/j.jmoldx.2014.02.001

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

- Discuss proficiency testing (PT) and its role in quality assurance.
- Understand methods-based proficiency testing (MBPT) as a subset of PT.
- Explain the changes observed in areas of medicine to which molecular diagnostic testing is applied given the adoption of sequenced-based clinical testing.
- Describe extraskeletal myxoid chondrosarcoma (EMC).
- Define the classification of EMC.
- Discuss the use of cytogenetics and molecular genetics as ancillary techniques to support an EMC diagnosis.
- Describe the limitations of antibody-based agglutination.
- Understand the advantages of DNA-typing assays for antigen screening.
- Discuss *BRCA1* and *BRCA2* germline mutations in hereditary breast/ovarian cancer (HBOC) syndrome.
- Understand the importance of *BRCA1* and *BRCA2* variants of uncertain significance (VUS).

1. Proficiency testing (PT) is intended to be an external measure of clinical laboratory quality. Based on the referenced Perspective, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:283-287.]

a. In the United States, PT is a requirement of accreditation by the Centers for Medicare and Medicaid Services.
b. PT is part of a quality assurance program to verify the efficiency and speed of laboratory testing.
c. Laboratories in the United States are certified under the Clinical Laboratory Improvement Amendments (CLIA) and accredited by professional organizations with deemed status, such as the College of American Pathologists (CAP).
d. Participation in external quality assessment (EQA) may be through CAP PT programs or through another proficiency testing provider accepted by CLIA.

Rationale: PT is part of a quality assurance program to verify the accuracy and reliability of laboratory testing.

2. Methods-based proficiency testing (MBPT) is a subset of overall PT and refers to an EQA approach that is based on method, rather than on each individual analyte tested. Based on the referenced Perspective, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 283-287.]

a. MBPT is already well established for several pathology subspecialty areas.
b. CAP offers a variety of PT products that are methods based, for example, in cytogenetics, flow cytometry, and immunohistochemistry.
c. The concept of MBPT complies with federal laboratory regulations.
d. MBPT in molecular diagnostics currently includes molecular cancer testing, in addition to inherited genetic conditions.

Rationale: Although MBPT in molecular diagnostics is currently limited to inherited genetic conditions, in the future, molecular cancer testing will probably include MBPT as well.

3. With the rapid adoption of sequenced-based clinical testing, the number of disease-causing variants in almost every area of medicine to which molecular diagnostic testing is applied is growing and is projected to expand. Based on the referenced Perspective, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 283-287.]

a. For inherited diseases, our earlier understanding of a single gene mutation for a single disease has evolved with the use of sequenced-based clinical testing.
b. In genome analysis, the number of variants per person is approximately three million.
c. For exomes, the number of variants per person approximates 50,000.
d. With the use of next generation sequencing (NGS), disease-causing mutations will outnumber the handful of genetic mutations that are currently typically tested in clinical laboratories.

Rationale: In genome analysis, the number of variants per person is approximately three million. For exomes, that number approximates 15,000 to 20,000.

4. Extraskeletal myxoid chondrosarcoma (EMC) is a rare mesenchymal tumor. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 314-323.]

a. EMC accounts for <10% of soft tissue sarcomas.
b. EMC rarely occurs in bone.
c. EMC is mainly a tumor of adults, with the median age in the sixth decade.
d. EMC is quite rare in children and adolescents.

Rationale: Extraskeletal myxoid chondrosarcoma (EMC) is a rare mesenchymal tumor that accounts for <3% of soft tissue sarcomas.

5. EMC has been considered a cartilaginous neoplasm because of some morphological findings that suggest chondroid differentiation. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 314-323.]

a. Generally, immunohistochemical studies are not helpful in establishing the diagnosis of EMC but may be useful to exclude other entities.
b. In contrast to other cartilaginous neoplasms, only a few EMCs actually express vimentin.
c. EMC is classified as a tumor of uncertain differentiation in the most recent edition of the *World Health Organization Classification of Tumors of Soft Tissue and Bone*.
d. On the basis of the results of immunohistochemical and ultrastructural investigations, EMC may have neural or neuroendocrine differentiation.

Rationale: Vimentin is the only marker consistently positive in EMC and is certainly not specific. In contrast to other cartilaginous neoplasms, only a few EMCs actually express S-100 protein and often with only focal to weak staining.

6. Cytogenetic and molecular genetic studies of EMC have found four pathogenetically relevant chromosome translocations. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 314-323.]

a. The most common translocation t(9;22)(q22;q12), found in approximately 25% of cases, results in a fusion of EWS RNA-binding protein 1 gene (*EWSR1*) at 22q12 to the nuclear receptor subfamily 4, group A, member 3 (*NR4A3*) gene at 9q22.
b. Fusion of the *EWSR1/NR4A3* chimeric transcripts produces different fusion variants, depending on the breakpoint in the genes.
c. The main variant of *EWSR1/NR4A3* chimeric transcripts is the type 1 fusion in which *EWSR1* exon 14 is fused to exon 4 of *NR4A3*.
d. The fusion protein of *EWSR1/NR4A3* chimeric transcripts consists of the NH2-terminal transactivation domain of EWSR1 linked to the entire NR4A3 protein.

Rationale: The most common translocation t(9;22)(q22;q12), found in approximately 75% of cases, results in a fusion of the *EWSR1* gene at 22q12 to the *NR4A3* gene at 9q22.

7. Germline deleterious mutations of *BRCA1* and *BRCA2* originate the hereditary breast/ovarian cancer (HBOC) syndrome. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:324-334.]

a. Carriers of the *BRCA1* and *BRCA2* inactivating mutations have an estimated lifetime risk of breast cancer of 65%, and the lifetime risk of ovarian cancer is 33% for *BRCA1* carriers and 50% for *BRCA2* carriers.
b. Although the magnitude of risk varies with the population and the study design, the finding of a deleterious germline mutation is crucial for the correct clinical management of HBOC families.
c. Finding a germline mutation allows identification of relatives who require increased surveillance and/or prophylactic interventions, as well as those who are not at increased cancer risk.
d. Genetic testing of the index case can identify variants of uncertain significance (VUS), also called unclassified variants, usually missense, silent and intronic variants or in-frame deletions and insertions.

Rationale: Carriers of *BRCA1* and *BRCA2* inactivating mutations have an estimated lifetime risk of breast cancer of 82%, and the lifetime risk of ovarian cancer is 54% for *BRCA1* carriers and 23% for *BRCA2* carriers.

8. *BRCA1* and *BRCA2* unclassified variants constitute a major problem for genetic counseling and follow-up of families with suspected HBOC syndrome. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:324-334.]

a. According to software analysis of splicing predictions, four variants that affect highly conserved regions of splicing induce disruption of intron-exon junctions.
b. *BRCA2* c.8488-1G>A causes the abolishment of the normal splice site and also generates a new splice site with a high score.
c. *BRCA1* c.4484G>T is a splicing mutation affecting the last nucleotide of *BRCA1* exon 14.
d. The *BRCA2* c.682-2A>C mutation affects the highly conserved region of the acceptor splice site, induces aberrant splicing with the production of two out-of-frame transcripts and segregates with the disease in the family, and is considered a pathogenic mutation.

Rationale: *BRCA2* c.8488-1G>A, like BRCA1 c.4484G>T, BRCA2 c.682-2A>C, and BRCA2 c.8954-5A>G, induces disruption of the intron-exon junction. *BRCA2* c.8954-5A>G causes the abolishment of the normal splice site and also generates a new splice site with a high score.

9. The intronic or exonic variants that are identified in the exon-intron boundaries of *BRCA1* and *BRCA2* may disrupt the splicing capacity, due to the highly conserved nature of these regions, but the effect is not predictable from genomic sequence alone. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:324-334.]

a. The *BRCA2* c.2T>G variant causes the disruption of the translation initiation codon.
b. In the Breast Cancer Information Core (BIC) database, *BRCA2* c.2T>G is described as clinically important, but no additional data is presented.
c. *BRCA2* c.2T>G abolishes the normal initiation codon of the BRCA2 protein and the closest ATG is in exon 2 leading to an in-frame protein.
d. The *BRCA2* c.2T>G variant was identified in a family with four affected members; it segregates in three of them and does not segregate in the daughter with breast cancer diagnosed at age 46 years.

Rationale: *BRCA2* c.2T>G abolishes the normal initiation codon of the BRCA2 protein and the closest ATG is in exon 4 (nucleotide 323 to 325), leading to an out-of-frame protein.

10. The standard method of phenotyping for red blood cell (RBC) antigens is antibody-based agglutination. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:335-342.]

a. Antibody-based agglutination involves long procedure duration.
b. Antibody-based agglutination involves limited range of antigen testing.
c. In the French Blood Service, blood donation qualification laboratories test all blood donations for ABO, Rhesus, KEL, MNS3, and MNS4.
d. Conventional hemagglutination is ill suited to high-throughput blood group phenotyping.

Rationale: In the French Blood Service, blood donation qualification laboratories test all blood donations for ABO, Rhesus, and KEL, but testing for other clinically significant antigens, including FY1, FY2, JK1, JK2, MNS3, and MNS4, is performed on only a fraction of donations, ie 5% to 10%.

11. Development of DNA-typing assays for antigen screening in blood donation qualification laboratories promises to enable blood banks to provide optimally matched donations. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:335-342.]

a. Many low-throughput methods allow point-by-point identification of single nucleotide polymorphisms (SNPs).
b. Low-throughput methods are unsuitable for large-scale genotyping and thus for routine blood donor screening.
c. Large-scale blood group genotyping is possible using several commercially available assays in various formats.
d. The currently available commercial assays are fully automated and allow simultaneous detection of a large number of SNPs in a single reaction.

Rationale: The currently available commercial assays allow simultaneous detection of a large number of SNPs in a single reaction but are not designed for fully automated use.

12. High-throughput DNA-typing could have a number of applications in blood banking. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:335-342.]

a. High-throughput DNA-typing would greatly facilitate support for chronically transfused patients at high risk of alloantibody production, such as patients with sickle cell disease, thalassemia, or autoimmune hemolytic anemia.
b. In association with conventional serological methods for detection of RBC antigens, routine DNA-based methods blood donor screening would improve transfusion safety by optimizing donor/recipient genocompatibilty.
c. For small batch production using the 96-well format system, the cost of genotyping, including genomic DNA extraction, labor, and equipment, is less than $1.20 per SNP for a multiplex set of 12 SNPs.
d. A drastic reduction in cost per SNP could be achieved by increasing the number of samples analyzed since the main expenses are microarray fabrication and nucleic acid extraction.

Rationale: For small batch production using the 96-well format system, the cost of genotyping, including genomic DNA extraction, labor, and equipment, is less than $2.60 per SNP for a multiplex set of eight SNPs.

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

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

A research article and related Commentary on the diagnosis of trypanosomatid infections and research articles on death-associated protein kinase I as a follicular lymphoma biomarker and the diagnostic potential of micro RNAs in malignant pleural mesothelioma were selected for the **July 2014 JMD CME Program in Molecular Diagnostics**. The authors of the referenced articles, the planning committee members, and staff have no relevant financial relationships with commercial interests to disclose.

Questions #1-4 are based on: González-Andrade P, Camara M, Ilboudo H, Bucheton B, Jamonneau V, Deborggraeve S: Diagnosis of trypanosomatid infections: Targeting the spliced leader RNA. J Mol Diagn 2014, 16:400-404; http://dx.doi.org/10.1016/j.jmoldx.2014.02.006 and related Commentary: Duncan R: Advancing molecular diagnostics for trypanosomatid parasites. J Mol Diagn 2014, 16:379-381; http://dx.doi.org/10.1016/j.jmoldx.2014.04.001

Questions #5-8 are based on: Andersen M, Grauslund M, Ravn J, Sørensen JB, Andersen CB, Santoni-Rugiu E: Diagnostic potential of miR-126, miR-143, miR-145, and miR-652 in malignant pleural mesothelioma. J Mol Diagn 2014, 16:418-430; http://dx.doi.org/10.1016/j.jmoldx.2014.03.002

Questions #9-12 are based on: Giachelia M, Bozzoli V, D'Alò F, Tisi MC, Massini G, Maiolo E, Guidi F, Cupelli E, Martini M, Larocca LM, Voso MT, Leone G, Hohaus S: Quantification of *DAPK1* promoter methylation in bone marrow and peripheral blood as a follicular lymphoma biomarker. J Mol Diagn 2014, 16:467-476; http://dx.doi.org/10.1016/j.jmoldx.2014.03.003

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

- Describe trypanosomatid genetics.
- Define the trypanosomatid spliced leader RNA (SL-RNA) molecule.
- Explain sleeping sickness.
- Define the epigenetic markers used for the evaluation of minimal residual disease in select cancers.
- Define and describe the clinical outcome of malignant pleural mesothelioma (MPM).
- Understand how MPM is diagnosed and treated.
- Define microRNAs (miRs) and their importance in cancer-related processes.
- Describe the clinical characteristics of follicular lymphoma (FL).
- Understand the *BCL2-IGH* rearrangement and its importance to the development of FL.
- Describe the role of epigenetic silencing of tumor suppressors in FL, particularly death-associated protein kinase 1 (*DAPK1)*.

1. Trypanosomatids are early eukaryotic protozoans that possess unique molecular features. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:400-404 and J Mol Diagn 2014, 16:379-381.]

a. Trypanosomatid genes typically do not harbor introns but are arranged in large polygenic clusters that are transcribed polycistronically.
b. Polycistronic pre-mRNA is processed into functional monocistronic mRNA molecules by trans-splicing and polyadenylation in trypanosomatids.
c. During trypanosomatid RNA maturation, a conserved spliced leader RNA (SL-RNA) molecule is donated to the 5' end of each individual mRNA.
d. The SL-RNA sequence of the trypanosomatids can be 20 to 35 nucleotides long.

Rationale: The SL-RNA sequence of the trypanosomatids can be 39 to 41 nucleotides long and carries methyl groups on the first four ribose moieties and on the first and fourth bases following the conserved 7-methylguanosine.

2. Among the trypanosomatids are three major human pathogens: *Trypanosoma brucei*, *T. cruzi*, and *Leishmania* spp. Based on the referenced article and related Commentary, select the ONE statement related to trypanosomatids that is NOT true: [See J Mol Diagn 2014, 16:400-404 and J Mol Diagn 2014, 16:379-381.]

a. mRNA is considered the best surrogate marker for viable organisms.
b. mRNA has a typical half-life of 10 minutes after death of the organism.
c. SL-RNA is a short, noncoding RNA sequence that is conserved, but unique, for each species.
d. SL-RNA is present in each mRNA molecule in the cell.

Rationale: mRNA is considered the best surrogate marker for viable organisms because it is rapidly degraded after death of the organism with a typical half-life of 3 minutes.

3. Trypanosomatid infections cause a series of devastating diseases in man and animals. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:400-404 and J Mol Diagn 2014, 16:379-381.]

a. Sleeping sickness is a devastating disease that is endemic in sub-Saharan Africa.
b. Sleeping sickness is caused by the *T. brucei* subspecies *gambiense* and *rhodesiense*.
c. *T. brucei rhodesiense* is associated with the chronic form of sleeping sickness in west and central Africa, whereas *T. brucei gambiense* causes acute sleeping sickness in east Africa.
d. The trypanosomes are transmitted to humans by tsetse flies and invade the brain.

Rationale: *T. brucei gambiense* is associated with the chronic form of sleeping sickness in west and central Africa, whereas *T. brucei rhodesiense* causes acute sleeping sickness in east Africa.

4. The diagnosis of infectious diseases has advanced significantly with the development of molecular diagnostics. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:400-404 and J Mol Diagn 2014, 16:379-381.]

a. Trypanosomatidae have developed mechanisms of gene expression that set them apart from all other eukaryotes.
b. The number of SL-RNA copies in a cell is at least 10,000.
c. The unique sequence of the SL makes its reverse-transcribed PCR amplification specific to the pathogen, only limited by the probability of a 39-base sequence occurring at random in the human genome.
d. The conservation of the SL sequence within the genera suggests that assays can be designed that could target multiple species.

Rationale: The number of SL-RNA copies in a cell is at least 8600, suggesting the potential for high sensitivity of detection of this multicopy target.

5. Malignant pleural mesothelioma (MPM) is an aggressive cancer originating from the mesothelial cells lining the pleura. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:418-430.]

a. Patients diagnosed with MPM typically have a history of long-term exposure to asbestos.
b. Patients diagnosed with MPM have a median survival of 24 months from the time of diagnosis.
c. Patients with MPM usually present with symptoms of pleural effusion (ie, chest pain and breathlessness).
d. Patients with MPM present less commonly with constitutional symptoms, such as weight loss and fatigue.

Rationale: Patients diagnosed with MPM typically have a history of long-term exposure to asbestos and poor prognosis with a median survival of 12 months from the time of diagnosis.

6. A major contributing factor to the poor prognosis of MPM is that symptoms generally occur at an advanced stage of the disease. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:418-430.]

a. MPM is often difficult to diagnose, which further hampers effective treatment.
b. Trimodal therapy is currently the preferred treatment modality consisting of chemotherapy, cytoreductive surgery, and radiotherapy.
c. The majority of MPM patients are eligible for trimodal therapy.
d. MPM is classified histologically as epithelioid, sarcomatoid, or biphasic subtypes, known to have better, worse, and intermediate prognosis, respectively.

Rationale: Only few (<20%) patients are eligible for trimodal therapy, which is currently the preferred treatment modality consisting of chemotherapy, cytoreductive surgery (extrapleural pneumonectomy or pleurectomy/decortication), and radiotherapy.

7. The main diagnostic criterion for MPM is deep invasion in the pleura and underlying fat tissue. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:418-430.]

a. Deep invasion in the pleura and underlying fat tissue can be easily demonstrated radiologically and histologically in small pleural biopsies.
b. Epithelioid MPM can be challenging to distinguish from reactive mesothelial hyperplasia.
c. Sarcomatoid MPM may resemble fibrous pleurisy.
d. At present, there are no accepted diagnostic biomarkers for MPM.

Rationale: Deep invasion in the pleura and underlying fat tissue is often difficult to demonstrate radiologically and histologically in small pleural biopsies.

8. MicroRNAs (miRs) are short non-coding RNAs that silence gene expression by base-pairing to complementary sequences primarily within the 3'-untranslated regions (3'-UTR) of target RNA. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:418-430.]

a. It is estimated that miRs target 3'-UTRs in nearly 50% of human mRNAs.
b. The latest version (release 19) of the miRBase database contains 2042 entries of mature human miRs.
c. miRs are known to regulate several fundamental cellular and cancer-related processes, including proliferation, apoptosis, invasion, metastasis, cell-cycle control, and metabolism.
d. miRs are particularly attractive as biomarkers in tissue samples processed for routine pathology.

Rationale: It is estimated that miRs target 3'-UTRs in more than 60% of human mRNAs.

9. Follicular lymphoma (FL) is the most common indolent type of non-Hodgkin lymphoma. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 467-476.]

a. The characteristic chromosomal translocation of FL, t(14;18)(q32;q31), is present in nearly 60% of cases.
b. The clinical course of FL is characterized by initial good responses to systemic therapies, including combinations of immunotherapy and chemotherapy.
c. The clinical course of FL is characterized by frequent relapses due to the inability of current treatment regimens to eradicate the neoplastic clone.
d. In addition to clinical characteristics, risk stratification takes advantage of genetic and molecular markers.

Rationale: The characteristic chromosomal translocation of FL, t(14;18)(q32;q31), is present in nearly 90% of cases.

10. A clinical predictor of outcome for FL is the FL International Prognostic Index (FLIPI), based on age, stage, hemoglobin, number of nodal site areas, and lactate dehydrogenase. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 467-476.]

a. The FLIPI has been improved by the development of the FLIPI2 score, based on age, bone marrow involvement, hemoglobin, diameter of the largest lymph node, and β_2-microglobulin.
b. The transposition of the *BCL2* oncogene to the regulatory region of the immunoglobulin heavy chain gene *IGH* leads to the down-regulation of *BCL2*.
c. The use of qualitative PCR allows a rapid detection of the chimeric *BCL2-IGH* rearrangement in up to 80% of cases.
d. Qualitative PCR is an important tool for the diagnostic workup and clinical follow-up of patients with FL.

Rationale: The transposition of the *BCL2* oncogene to the regulatory region of the immunoglobulin heavy chain gene *IGH* leads to the up-regulation of the anti-apoptotic protein *BCL2*.

11. In addition to genetic alterations, epigenetic modifications play an important role in lymphomagenesis. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 467-476.]

a. The epigenetic silencing of tumor suppressor genes is considered a common event in the early stage of FL transformation.
b. Epigenetic silencing targets cell cycle inhibitors p15 (*CDKN2B*), p16 (*CDKN2A*), and p57 (*CDKN1C*) and the pro-apoptotic gene death-associated protein kinase 1 (*DAPK1*) in FL.
c. Promoter hypermethylation of *DAPK1* has been detected in 50% of FL.
d. *DAPK1* is among the 10 genes with the most significant increase in methylation in FL.

Rationale: Promoter hypermethylation of *DAPK1* has been detected in 85% of FL.

12. The development of quantitative methods that allow specific and sensitive detection of aberrant DNA methylation has increased interest in exploring epigenetic markers for the study of minimal residual disease. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 467-476.]

a. The epigenetic markers used for the evaluation of minimal residual disease include *ESR1* and p15 in acute myeloid leukemia.
b. In acute lymphoblastic leukemia, the epigenetic markers used for the evaluation of minimal residual disease include *TP73*, p15, and p57.
c. The epigenetic marker used for the evaluation of minimal residual disease in diffuse large B-cell lymphoma includes p57.
d. The epigenetic marker used for the evaluation of minimal residual disease includes *DAPK1* in neuroblastoma.

Rationale: The epigenetic marker used for the evaluation of minimal residual disease includes Ras-associated domain family 1, isoform A in neuroblastoma.

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

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

A Technical Advance article on noncontinuously binding loop-out primers and three research articles on ultrasensitive human DNA detection, epidermal growth factor receptor (*EGFR*) mutation detection in cerebrospinal fluid, and molecular diagnosis of invasive aspergillosis were selected for the **September 2014 JMD CME Program in Molecular Diagnostics**. The authors of the referenced articles, the planning committee members, and staff have no relevant financial relationships with commercial interests to disclose.

Questions #1-2 are based on: Sumner K, Swensen JJ, Procter M, Jama M, Wooderchak-Donahue W, Lewis T, Fong M, Hubley L, Schwarz M, Ha Y, Paul E, Brulotte B, Lyon E, Bayrak-Toydemir P, Mao R, Pont-Kingdon G, Best DH: Noncontinuously binding loop-out primers for avoiding problematic DNA sequences in polymerase chain reaction and Sanger sequencing. J Mol Diagn 2014, 16:477-480; http://dx.doi.org/10.1016/j.jmoldx.2014.04.005

Questions #3-6 are based on: Debeljak M, Freed DN, Welch JA, Haley L, Beierl K, Iglehart A, Pallavajjala A, Gocke CD, Leffell MS, Lin MT, Pevsner J, Wheelan S, Eshleman JR: Haplotype counting by next generation sequencing for ultrasensitive human DNA detection. J Mol Diagn 2014, 16:495-503; http://dx.doi.org/10.1016/j.jmoldx.2014.04.003

Questions #7-9 are based on: Yang H, Cai L, Zhang Y, Tan H, Deng Q, Zhao M, Xu X: Sensitive detection of *EGFR* mutations in cerebrospinal fluid from lung adenocarcinoma patients with brain metastases. J Mol Diagn 2014, 16:558-563; http://dx.doi.org/10.1016/j.jmoldx.2014.04.008

Questions #10-12 are based on: Wang L, He Y, Xia Y, Su X, Wang H, Liang S: Retrospective comparison of NASBA, real-time PCR, and galactomannan test for diagnosis of invasive aspergillosis. J Mol Diagn 2014, 16:584-590; http://dx.doi.org/10.1016/j.jmoldx.2014.05.001

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

- Understand that genetic polymorphisms are a potential source of genotyping errors.
- Describe noncontinuously binding (loop-out) oligonucleotide hybridization probes.
- Explain the advantages of non-myeloablative conditioning regimens.
- Define and understand short tandem repeats (STRs) and STR analysis.
- Describe single nucleotide polymorphisms (SNPs).
- Understand that brain metastases are a frequent complication of non-small cell lung cancer (NSCLC).
- Describe the importance of identifying biomarkers in cerebrospinal fluid (CSF) in patients with brain metastases.
- Understand epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitors (TKI).
- Define invasive aspergillosis (IA).
- Describe galactomannan (GM) and how it is measured.
- Explain nucleic acid sequenced-based amplification (NASBA).

1. Genetic polymorphisms are a potential source of genotyping errors. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:477-480.]

a. Sequence variants that occur in PCR primer binding sites can cause allele-specific PCR dropout, leading to false-negative or apparent homozygous results.
b. A polymorphism in intron 2 of *MEN1*, c.-23-16C>G (rs509606), has an allele frequency of approximately 35% in white Europeans.
c. Although rs509606 lies outside the PCR primer binding sites, the allele containing this variant preferentially amplifies in heterozygous individuals, resulting in dropout of the wild-type allele.
d. Amplification bias in favor of rs509606 has been reported to occur because rs509606 changes the stability of G-quadruplex- and i-motif-like DNA secondary structures in the amplicon.

Rationale: A polymorphism in intron 2 of *MEN1*, c.-23-16C>G (rs509606), has an allele frequency of approximately 18% in white Europeans.

2. Noncontinuously binding (loop-out) oligonucleotide hybridization probes have been described for molecular haplotyping and multiplex genotyping of nonadjacent sequence variants. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:477-480.]

a. In loop-out probes, stretches of nucleotides are omitted between two or more nearby regions to be tested.
b. Flanking segments of eight nucleotides on either side of the omitted sequence are sufficient for loop-out probes to reliably bind to multiple regions of interest.
c. Additional PCR and sequencing reactions increase the overall costs of running a test.
d. Using a modified PCR or additives for a single reaction is disruptive to the clinical laboratory workflow.

Rationale: Flanking segments of 11 nucleotides on either side of the omitted sequence are sufficient for loop-out probes to reliably bind to multiple regions of interest.

3. Myeloablative conditioning and allogeneic stem cell transplantation (alloSCT) have historically been limited to the treatment of lethal hematologic malignancies in children or young adults. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:495-503.]

a. The advent of highly immunosuppressive, non-myeloablative regimens has expanded the clinical use of alloSCT to include middle-aged, fit patients with hematologic malignancies.
b. AlloSCT is used for patients with non-malignant disorders, such as sickle cell disease (SCD).
c. Non-myeloablative conditioning regimens offer the additional safeguard of recovery of autologous hematopoiesis in the event of graft rejection.
d. Non-myeloablative conditioning regimens may be a safer option in patients at risk for immune-mediated rejection of the donor graft.

Rationale: The advent of highly immunosuppressive, non-myeloablative regimens has expanded the clinical use of alloSCT to include older, less fit patients with hematologic malignancies.

4. Chimerism testing at set intervals is an effective method for detecting graft rejection or recurrence of the original hematopoietic neoplasm after allogeneic hematopoietic stem cell transplantation (HSCT). Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:495-503.]

a. Decades ago, bone marrow engraftment monitoring was performed using Southern blotting and minisatellite or variable number of tandem repeats loci.
b. Today, short tandem repeat (STR) loci are most commonly used for monitoring bone marrow engraftment.
c. Each STR unit is 1 to 3 bases in length.
d. STRs are composed of 10 to 60 tandemly repeated units.

Rationale: STRs are composed of 10 to 60 tandemly repeated units, in which each unit is 1 to 6 bases in length.

5. Human identity testing is critical to the fields of forensics, paternity, and hematopoietic stem cell transplantation. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:495-503.]

a. STRs are widely distributed throughout the human genome.
b. STRs are highly variable between individuals, and therefore allow for excellent differentiation between individuals, including patient and donor, even if they are closely related.
c. Most laboratories use multiplex PCR based kits, originally developed for forensics analysis using Combined DNA Index System (CODIS) loci.
d. STR analysis always involves PCR amplification using fluorescently labeled primers followed by amplicon separation by polyacrylamide gel electrophoresis.

Rationale: STR analysis most commonly involves PCR amplification using fluorescently labeled primers followed by amplicon separation by capillary electrophoresis.

6. STRs and single nucleotide polymorphisms (SNPs) can be used to monitor bone marrow engraftment. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:495-503.]

a. SNPs are theoretically superior to STR-based analysis because analysis of STR loci by capillary electrophoresis is relatively insensitive (limit of detection 5 to 10%).
b. Microsatellite alleles of varying length amplify with different efficiencies, thus making them inherently biased.
c. STR amplification can be difficult in the setting of highly degraded DNA.
d. SNPs are less attractive as targets due to their inherently lower informativity, requiring many more SNPs to be tested to identify those that distinguish donor from recipient.

Rationale: SNPs are theoretically superior to STR based analysis because analysis of STR loci by capillary electrophoresis is relatively insensitive (limit of detection 1 to 5%) and microsatellite alleles of varying length amplify with different efficiencies, thus making them inherently biased.

7. Brain metastases are a frequent complication of non-small cell lung cancer (NSCLC), especially in patients with lung adenocarcinoma. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:558-563.]

a. Brain metastases are observed in 50% to 75% of patients at initial diagnosis of NSCLC.
b. Patients with metastases are unable to undergo surgical resection of primary or cranial metastatic tumors to provide specimens for histopathological or biomarker studies.
c. Tumor-derived DNA can be secreted into body fluids surrounding the tumor.
d. The development of methods to identify potential molecular biomarkers from nonsurgical biopsy samples, such as cerebrospinal fluid (CSF), may facilitate the identification of clinically relevant gene signatures in patients with metastatic brain tumors.

Rationale: Brain metastases are a frequent complication of non-small cell lung cancer (NSCLC), especially in patients with lung adenocarcinoma. Brain metastases are observed in 30% to 50% of patients at initial diagnosis, with more patients developing metastases during treatment.

8. Brain metastases are diagnosed according to clinical presentation, primary malignant tumor, and radiological imaging. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:558-563.]

a. If the computed tomography or magnetic resonance imaging (MRI) aspect is atypical, tissue diagnosis, including brain tumor or CSF cytology, is necessary.
b. In certain clinical situations, MRI would not be helpful for patients with leptomeningeal metastases in which positive CSF cytology results are <70%.
c. The detection of oncogenes in CSF might facilitate the diagnosis of brain metastases in patients with lung adenocarcinoma.
d. The detection of epidermal growth factor receptor (*EGFR*) gene status in tumor-derived free DNA in CSF might be a good clinical option.

Rationale: In certain clinical situations, MRI would not be helpful for patients with leptomeningeal metastases in which positive CSF cytology results are <40%.

9. *EGFR* tyrosine kinase inhibitor (TKI) is a small-molecular agent capable of penetrating brain tissue. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:558-563.]

a. *EGFR*-TKI has been found to significantly improve survival rates and tumor responses in lung adenocarcinoma patients with metastatic brain tumors that harbor *EGFR*-activating mutations.
b. The most common target populations for treatment with *EGFR*-TKI are males with adenocarcinomas.
c. *EGFR* gene status can only be detected in approximately 10% of patients with advanced NSCLC in China.
d. The limited availability of testing technology and economic factors are the leading causes of the low detection rate of NSCLC in China.

Rationale: The most common target populations for treatment with *EGFR*-TKI are females with adenocarcinomas, because these patients have a higher rate of *EGFR* mutation.

10. Invasive aspergillosis (IA), an opportunistic fungal infection, has been increasingly recognized as a major cause of morbidity and mortality in immunocompromised patients. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:584-590.]

a. IA remains difficult to diagnose despite great advances in imaging and antigen-based serological detection.
b. Because early diagnosis is important for improved outcomes, efforts have been devoted to develop diagnostic assays targeting fungal biomarkers that offer the potential for new paradigms in prevention and early treatment of IA.
c. Galactomannan (GM), a fungal biomarker, can be released into the serum and bronchoalveolar lavage (BAL) fluid during fungal infection.
d. GM is a polysaccharide component of the fungal nuclear membrane.

Rationale: One of the most attractive fungal biomarkers is GM, a polysaccharide component of the fungal cell wall, which can be released into the serum and BAL fluid during fungal infection.

11. Measurement of GM can be achieved by using a commercially available ELISA kit, which has been approved by the US Food and Drug Administration. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:584-590.]

a. False-negative results with the GM ELISA assay kit may occur because of cross-reactivity with nutritional preparations.
b. The GM ELISA assay lacks species-specificity and is unable to differentiate among *Aspergillus* spp.
c. With the advent of PCR, it has become possible to detect pathogen genes in clinical samples, allowing early diagnosis of IA.
d. Conventional PCR and real-time quantitative PCR (qPCR) amplification techniques lack standardization and clinical validation for IA.

Rationale: False-positive results with the GM ELISA assay kit may occur because of cross-reactivity with certain antibiotics or parenteral nutrition preparations.

12. Nucleic acid sequenced-based amplification (NASBA) is a RNA-directed isothermal transcription-based amplification process that specifically amplifies RNA even in the presence of genomic DNA. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:584-590.]

a. The amplification efficiency of NASBA has been shown to be more robust than PCR.
b. Amplification using NASBA yields $>10^{10}$ amplicons in 45 minutes.
c. The advantages of NASBA over PCR (simplicity, speed, and sensitivity) have stimulated interest in evaluating its ability to detect *Aspergillus* RNA in clinical samples.
d. The authors measured circulating *Aspergillus* GM, DNA, and RNA in blood samples of 80 patients; the data support the great potential of NASBA and qPCR, singly or in combination, for diagnosis of IA in high-risk populations.

Rationale: The amplification efficiency of NASBA has been shown to be more robust than PCR, yielding $>10^{12}$ amplicons in as little as 30 minutes.

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

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

A Review on molecular oncology testing in developing nations and research articles on extra alleles in fragile X testing and long noncoding RNAs as putative biomarkers for prostate cancer were selected for the **November 2014 JMD CME Program in Molecular Diagnostics**. The authors of the referenced articles, the planning committee members, and staff have no relevant financial relationships with commercial interests to disclose.

Questions #1-7 are based on: Gulley ML, Morgan DR: Molecular oncology testing in resource-limited settings. J Mol Diagn 2014, 16:601-611; http://dx.doi.org/10.1016/j.jmoldx.2014.07.002

Questions #8-9 are based on: Lee B, Mazar J, Aftab MN, Qi F, Shelley J, Li J, Govindarajan S, Valerio F, Rivera I, Thurn T, Tran TA, Kameh D, Patel V, Perera RJ: Long noncoding RNAs as putative biomarkers for prostate cancer detection. J Mol Diagn 2014, 16:615-626; http://dx.doi.org/10.1016/j.jmoldx.2014.06.009

Questions #10-12 are based on: Wakeling EN, Nahhas FA, Feldman GL: Extra alleles in *FMR1* triple-primed PCR: Artifact, aneuploidy, or somatic mosaicism? J Mol Diagn 2014, 16:689-696; http://dx.doi.org/10.1016/j.jmoldx.2014.06.006

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

- Understand the annual global incidence of cancer.
- Describe the infectious agents that may cause some cancer-related deaths in developing nations.
- Explain available tests that are devised specifically for resource-poor areas.
- Understand hepatitis B virus (HBV) and hepatitis C virus (HCV) infections.
- Describe tuberculosis and the select molecular tests that can detect it.
- Explain the Cancer Genome Atlas (TCGA) project.
- Describe portable test systems that can be used in the military.
- Understand prostate cancer (PCa) incidence and prostate-specific antigen (PSA) testing.
- Describe long noncoding RNAs (lncRNAs).
- Define Fragile X syndrome (FXS) and how it affects males and females.
- Explain the cause of FXS.
- Describe the characteristics of FXS premutation carriers.

1. Modern technology may address gaps in healthcare through rapid inexpensive automated test systems that identify and monitor the types of neoplasia prevalent in resource-poor areas. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:601-611.]

a. Noncommunicable diseases are projected to become the major global health burden in the near term, with cancer accounting for approximately one-quarter of this burden, of which, at least one-third is preventable.
b. The annual global incidence of cancer is projected to increase from 5.2 to 15.4 million by 2030, with 7.5 million expected deaths.
c. Over two-thirds of the burden will occur in low- and middle-income countries, wherein seven cancer types (lung, colon, breast, stomach, liver, cervical, esophageal) account for nearly two-thirds of incidents.
d. In the world's poorest countries compared to developed nations, women are more than twice as likely to die of their breast cancer, and children are up to 9-fold less likely to be cured of acute lymphoblastic leukemia.

Rationale: The annual global incidence of cancer is projected to increase from 12.7 to 22.2 million by 2030, with 13.1 million expected deaths.

2. In developing nations, nearly one-quarter of cancers are infection related. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 601-611.]

a. Four infectious agents account for over 80% of the burden: human papillomavirus (HPV), *Helicobacter pylori*, hepatitis B virus (HBV), and hepatitis C virus (HCV).
b. Epstein-Barr virus (EBV) adds a significant burden in several areas of the world.
c. Approximately 70% of infection-associated cancers occur in people under age 50.
d. Compared to more developed regions, less developed nations have more cancers of the stomach, uterine cervix, and liver, all three of which are infection-related.

Rationale: Approximately 30% of infection-associated cancers occur in people under age 50.

3. Rapid low-cost devices are being developed to test for HPV DNA and RNA. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 601-611.]

a. A commercially designed test system has been devised specifically for resource-poor areas, and published data suggest that its performance is similar to a US Food and Drug Administration (FDA)–approved test.
b. The battery-powered bench top instrument requires neither electricity nor running water to perform hybrid capture of RNA probes bound to high-risk HPV DNA genomes.
c. The manufacturer states that the reagents are tolerant of temperature swings that may characterize a rural laboratory having spotty electricity for refrigeration.
d. The test can be performed by minimally trained technologists at 6-fold less cost and 3-fold less time than an FDA-approved assay, potentially permitting same-day intervention for HPV-positive patients.

Rationale: The test can be performed by minimally trained technologists at 3-fold less cost and 6-fold less time than an FDA-approved assay, potentially permitting same-day intervention for HPV-positive patients.

4. HBV and HCV infections are common and predispose to hepatocellular carcinoma. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 601-611.]

a. Transfusion-mediated infection remains a concerning means of spread for HBV, HCV, and other pathogens in countries lacking a centralized system for blood collection and laboratory testing for transmissible agents.
b. Perinatal infection accounts for about half of the burden among the 500 million people with chronic HBV infection.
c. Vaccination is recommended for HBV-related cancer prevention, whereas treatment of HCV is associated with reduced cancer risk.
d. Viral genomes can be detected, characterized, and monitored using molecular tests, such as real-time quantitative PCR.

Rationale: Perinatal infection accounts for about half of the burden among the 350 million people with chronic HBV infection.

5. Tuberculosis is responsible for 1.7 million deaths per year. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 601-611.]

a. The risk of lung cancer increases 18-fold among tuberculosis patients.
b. Proposed mechanisms of mycobacteria-related carcinogenesis include long-term immune stimulation, neoangiogenesis, and DNA damage from reactive oxygen species.
c. Molecular tests can detect and speciate mycobacteria as well as predict drug resistance to assist clinicians in selecting rifampin, isoniazid, or second-line medications.
d. A PCR test system specifically designed to be rapid and user-friendly in low-volume testing laboratories has been developed to help guide appropriate therapy in tuberculosis-endemic regions.

Rationale: The risk of lung cancer increases 11-fold among tuberculosis patients.

6. The Cancer Genome Atlas (TCGA) project is a major step forward in cataloging mutation patterns and gene expression profiles in concert with traditional diagnostic histopathology. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 601-611.]

a. TCGA results to date confirm known cancer-related infections and provide new insights into the genetic underpinnings of neoplasia.
b. Multigene test panels can characterize signaling pathways driving tumor growth.
c. Genotyping is achievable on fresh, frozen, or fixed tissue in 30 minutes using an instrument platform that performs automated extraction followed by analysis to query 25 mutations in *KRAS*, *BRAF*, and *PIK3CA* genes.

d. A more practical test panel in hospitals lacking on-site pathology services might examine fine needle aspirate material from a mass lesion to help distinguish infection from tumor pending send out for a pathologist's definitive diagnosis days to weeks later.

Rationale: A proof-of-principle study showed that genotyping is achievable on fresh, frozen, or fixed tissue in just 70 minutes using an instrument platform that performs automated extraction, followed by analysis to query 13 mutations in *KRAS*, *BRAF*, and *PIK3CA* genes.

7. Government-sponsored research has devised test systems for military and aerospace use, and some of these advances have been adapted for the benefit of civilian healthcare facilities. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16: 601-611.]

a. In the 1990s, a briefcase-bound version of a thermocycler was developed that was later redesigned for clinical laboratory use.
b. Currently, a system is being developed to test 15 nucleic acid targets in two hours with only 5 minutes of hands-on time.
c. These devices tend to operate without electricity and have flexible test options with barcode readers to assure proper selection reagents for each protocol.
d. Reagents are freeze dried, which makes them light weight to transport, and stable at room temperature for six months.

Rationale: A system is being developed to test 27 nucleic acid targets in one hour with only 2 minutes of hands-on time.

8. Despite advances associated with modern medicine, improvements in prostate cancer (PCa)-related mortality have been marginal at best. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:615-626.]

a. According to the 2013 National Cancer Institute estimates, there will be 290,720 new PCa diagnoses this year; for 23,590 patients, it is likely to be fatal.
b. Most men with PCa have indolent disease for which conservative therapy or an active surveillance approach would be more appropriate and would result in less treatment-related morbidity.
c. A contributing problem has been the widespread use of prostate-specific antigen (PSA) testing.
d. The PSA test has low specificity for cancer and cannot differentiate indolent and aggressive cancers.

Rationale: According to the 2013 National Cancer Institute estimates, there will be 238,590 new prostate cancer (PCa) diagnoses this year; for 29,720 patients, it is likely to be fatal.

9. Long noncoding RNAs (lncRNAs) are RNA transcripts >200 nucleotides in length. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:615-626.]

a. lncRNAs exhibit cell type–specific expression and are localized to specific subcellular compartments.
b. A number of lncRNAs are known to play important roles during cellular development and differentiation.
c. Like microRNAs (miRNAs), lncRNAs are dysregulated in various medical conditions.
d. lncRNA AK024556 (*SPRY4-IT1*) is up-regulated in human PCa, and siRNA-mediated knock-down of *SPRY4-IT1* in PCa cells alters cellular growth and differentiation and increases the rate of apoptosis.

Rationale: lncRNA AK024556 (*SPRY4-IT1*) is up-regulated in human melanomas, and siRNA-mediated knock-down of *SPRY4-IT1* in melanoma cells alters cellular growth and differentiation and increases the rate of apoptosis.

10. Fragile X syndrome (FXS) is the most common inherited form of intellectual disability. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:689-696.]

a. FXS affects 1:3000 to 1:4000 females and 1:6000 to 1:8000 males.
b. Males with FXS have moderate to severe intellectual disabilities, behavioral difficulties, macroorchidism, and characteristic facial dysmorphism.
c. FXS females are usually more mildly affected than males.
d. Females with FXS exhibit normal to mildly impaired intellect, learning difficulties, and emotional problems, including depression and anxiety disorders.

Rationale: FXS affects 1:3000 to 1:4000 males and 1:6000 to 1:8000 females.

11. FXS is caused by loss of expression of the *FMR1* gene located on chromosome Xq27.3. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:689-696.]

a. *FMR1* encodes the fragile X mental retardation protein (FMRP).
b. FMRP is a RNA-binding protein that is highly expressed in neurons.

c. The stability, subcellular localization, and translation of several mRNAs involved in synaptic structure and function are regulated by FMRP.
d. Approximately 70% of FXS cases result from a CGG repeat expansion in the 5' untranslated region (UTR) of *FMR1*.

Rationale: Greater than 98% of FXS cases result from a CGG repeat expansion in the 5' UTR of *FMR1*.

12. Premutation alleles (55 to 200 CGG repeats in the *FMR1* gene) are particularly unstable in female meiosis with repeats as small as 56 and 59 CGGs expanding to full mutations in one generation. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:689-696.]

a. Approximately 1:151 to 1:178 females and 1:468 males in the U.S. are premutation carriers.
b. In addition to the significant risk of having a child with a full mutation, premutation carriers are also at risk of developing other *FMR1*-related disorders.
c. Up to 50% of female carriers will develop fragile X primary ovarian insufficiency, defined as cessation of menses by age 49.
d. Fragile X–associated tremor ataxia syndrome is a late-onset progressive cerebellar ataxia with intention tremor that affects 30% of male and 8% to 16.5% of female premutation carriers over age 50.

Rationale: Up to 20% of female carriers will develop fragile X primary ovarian insufficiency, defined as cessation of menses by age 40.

American Society for Investigative Pathology
9650 Rockville Pike
Bethesda, MD 20814 (USA)
Tel: 301-634-7130
Fax: 301-634-7990
Email: journalcme@asip.org
www.asip.org/CME/