JMD 2012 CME PROGRAM

IN MOLECULAR DIAGNOSITCS

Based on JMD Volume 14

(January, March, May 2012)

(July, September, November 2012)

ANSWER BOOKLET

American Society for Investigative Pathology
Investigating the Pathogenesis of Disease

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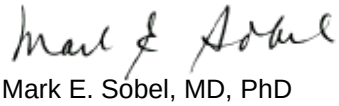
ASIP 2012 JMD CME PROGRAM IN MOLECULAR DIAGNOSTICS

Dear Colleague,

In 2012, ASIP organized its annual Journal CME Program based on selected articles in *The Journal of Molecular Diagnostics* (*JMD*) into two semi-annual programs because different sponsors were used for CME accreditation. The *JMD* CME Program in Molecular Diagnostics for January, March, and May was ACCME-accredited through the joint sponsorship of ASIP and FASEB (Federation of American Societies for Experimental Biology). The *JMD* CME Program in Molecular Diagnostics for July, September, and November was ACCME-accredited through the joint sponsorship of ASIP and ASCP (American Society for Clinical Pathology).

The first half of this Answer Booklet includes the CME exams for January, March, and May issues of *JMD*, Volume 14. The CME exams for the second half of the year (July, September, November issues of *JMD*, Volume 14) are included in the second section.

Sincerely yours,



Mark E. Sobel, MD, PhD
Executive Officer
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ASIP Semiannual 2012 JMD CME Program in Molecular Diagnostics

[January, March, May issues of JMD Volume 14]

CME Accreditation Statement: This activity ("ASIP Semiannual 2012 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 Federation of American Societies for Experimental Biology (FASEB) and the American Society for Investigative Pathology (ASIP). FASEB is accredited by the ACCME to provide continuing medical education for physicians.

FASEB designates this journal-based CME activity ("ASIP Semiannual 2012 JMD CME Program in Molecular Diagnostics") for a maximum of 24 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

American Society for Investigative Pathology

CONTINUING MEDICAL EDUCATION (CME)

This activity ("ASIP Semiannual 2012 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 Federation of American Societies for Experimental Biology (FASEB) and the American Society for Investigative Pathology (ASIP). FASEB is accredited by the ACCME to provide continuing medical education for physicians.

FASEB designates this journal-based CME activity ("ASIP Semiannual 2012 JMD CME Program in Molecular Diagnostics") for a maximum of 24 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

The ASIP Semiannual 2012 JMD CME Program in Molecular Diagnostics is a semiannual program consisting of a series of at least 24 questions based on selected articles in the January – May 2012 issues (Volume 14 Numbers 1-3) 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 July 31, 2012 to receive CME credit. Participants will earn 8 *AMA PRA Category 1 Credit(s)*™ for successful completion of each exam.

Should you have questions, contact the ASIP Education Office (301-634-7940; email journalCME@asip.org), 9650 Rockville Pike, Bethesda, MD 20814 or the FASEB Office of Scientific Meetings and Conferences (301-634-7010; email fasebcme@faseb.org).

SAM Credit

The ASIP 2012 Semiannual JMD CME Program in Molecular Diagnostics is approved by the American Board of Pathology for up to 24 SAM credits. Physicians should only claim credit commensurate with the extent of their participation in the activity. After successfully completing the CME exams as described above, participants may separately apply for SAM credit by completing SAM applications online on the ASIP website (www.asip.org). All SAM applications must be completed by December 31, 2012 for participants to receive SAM credit.

Should you have questions about SAM credits, contact the ASIP Education Office (301-634-7940; email journalCME@asip.org), 9650 Rockville Pike, Bethesda, MD 20814.

Meeting Objective/Target Audience

The objective of the ASIP Semiannual 2012 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 ASIP Semiannual 2012 JMD CME Program in Molecular Diagnostics is designed to meet the participants' education needs in the physician competency area of Medical Knowledge, as defined by the Accreditation Council for Graduate Medical Education (ACGME) and the American Board of Medical Specialties (ABMS), and to support participants' lifelong learning towards a goal of promoting patient safety and improving patient care and is specifically targeted to trainees, clinicians and researchers investigating mechanisms of disease who wish to advance their knowledge of the cellular and molecular biology of disease.

Educational Objectives
At the completion of the ASIP Semiannual 2012 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 Policy
The Federation requires that participants in FASEB-sponsored educational programs be informed of the organizers' and the presenters' (speaker, faculty, author, or contributor) academic and professional affiliation, and the existence of any relevant financial relationship an organizer or a presenter has with any proprietary entity producing health care goods or services consumed by, or used on patients, with the exemption of non-profit or government organizations and non-health care related companies. The intent of this disclosure is not to prevent a presenter from providing educational content but allows the participant to be fully knowledgeable in evaluating the information being presented.

Disclosure includes any relationship that may bias one's presentation or which, if known, could give the perception of bias. These situations may include, but are not limited to: 1) stock options or bond holdings in a for-profit corporation or self-directed pension plan; 2) research grants; 3) employment (full or part-time); 4) ownership or partnership; 5) consulting fees or other remuneration; 6) non-remunerative positions of influence such as officer, board member, trustee, or public spokesperson; 7) receipt of royalties; 8) speaker's bureau; 9) other. For full-time employees of industry or government, the affiliation listed in the Program/Article will constitute full disclosure.

None of the organizers of this educational activity disclosed a relevant financial relationship. Relevant financial relationships of the authors of selected articles in this journal CME program will be disclosed in a footnote to the article and in each examination.

ASIP 2012 Journal CME Programs

ASIP Semiannual 2012 JMD CME Program in Molecular Diagnostics

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

***The Journal of Molecular Diagnostics*, Volume 14, Number 1 (January 2012)**

www.asip.org/CME/journalCME.htm

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

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

1. Advances in technology and quality assurance suggest that genomic profiling is now reliable enough for medical decision making in clinical trials. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:1-11; DOI: 10.1016/.jmoldx.2011.09.003; Margaret M. Gulley is a consultant for McKesson, Roche Molecular Systems, and Abbott Laboratories and serves on the clinical advisory board of Generation Health; none of the other authors of the referenced article disclosed any relevant financial relationships.]

a. RNA profiles that are unique to clinical status can assist with diagnosis, prognosis, monitoring, and predicting efficacy of therapy.
b. The FDA has cleared or approved RNA-based assays to detect HIV, hepatitis C virus, influenza virus, and mycobacterium tuberculosis.
c. Of the 10 multianalyte RNA assays that have been FDA cleared, six are respiratory virus panels.
d. According to the American Medical Association Current Procedural Terminology Editorial Panel, the minimum number of analytes comprising an array is 18, although some multiplexed tests target thousands of RNAs at once.

Rationale: According to the American Medical Association Current Procedural Terminology Editorial Panel, the minimum number of analytes comprising an array is 11.

2. RNA rapidly degrades unless special precautions are taken to preserve it. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:1-11; DOI: 10.1016/.jmoldx.2011.09.003; Margaret M. Gulley is a consultant for McKesson, Roche Molecular Systems, and Abbott Laboratories and serves on the clinical advisory board of Generation Health; none of the other authors of the referenced article disclosed any relevant financial relationships.]

a. Formalin functions by aldehyde cross-linking to generate a scaffold preventing tissue degradation and diminishing the activity of unwanted RNases.
b. Diffusion of formalin into tissue is a function of distance and density.
c. Formalin fixation between 2 and 6 hours was ideal with respect to downstream RNA quality.
d. Overfixation with 10% neutral-buffered formalin hardens tissue and increases cross-links with macromolecules, rendering intact RNA difficult to recover.

Rationale: Fixation between 4 and 48 hours was reasonable, although 12 to 24 hours was ideal with respect to downstream RNA quality.

3. Non-formalin fixatives often yield high-quality RNA. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:1-11; DOI: 10.1016/.jmoldx.2011.09.003; Margaret M. Gulley is a consultant for McKesson, Roche Molecular Systems, and Abbott Laboratories and serves on the clinical advisory board of Generation Health; none of the other authors of the referenced article disclosed any relevant financial relationships.]

a. In stored blood, RNA profiles showed more degradation in TRIzol-preserved cells than in frozen cells.
b. Stabilization of RNA at the bedside is feasible using commercial blood collection systems that must be validated for their intended use.
c. Alcohol-based solutions often yield high-quality RNA but provide less histological detail compared to formalin.
d. Alcohol-based solutions can have an adverse impact on immunostaining.

Rationale: In stored blood, RNA profiles for frozen versus TRIzol-preserved cells were similar.

4. It is often worth evaluating RNA quantity and quality before subjecting a specimen to expensive microarray analysis. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:1-11; DOI: 10.1016/.jmoldx.2011.09.003; Margaret M. Gulley is a consultant for McKesson, Roche Molecular Systems, and Abbott Laboratories and serves on the clinical advisory board of Generation Health; none of the other authors of the referenced article disclosed any relevant financial relationships.]

a. RNA concentration is measurable by UV spectrophotometry or fluorimetry.
b. RNA integrity scores predict amplifiability from paraffin tissue blocks.
c. The spectrum of RNA size is dramatically larger in fresh or frozen tissue compared with formalin-fixed, paraffin-embedded tissue.
d. Although a 1-hour delay in fresh specimen processing does not adversely affect the RNA integrity score, it may disturb individual analytes.

Rationale: By quantitative RT-PCR (RT-qPCR), amplicons >500 bp are infrequently achieved from formalin-fixed tissues compared with frozen tissue. RNA integrity scores do not predict amplifiability from paraffin tissue blocks. Assessing levels of housekeeping transcripts is helpful for assessing specimen quality.

5. Quality control is among the most important quality assurance measures. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:1-11; DOI: 10.1016/.jmoldx.2011.09.003; Margaret M. Gulley is a consultant for McKesson, Roche Molecular Systems, and Abbott Laboratories and serves on the clinical advisory board of Generation Health; none of the other authors of the referenced article disclosed any relevant financial relationships.]

a. An endogenous control checks an inherent feature of a patient sample, such as levels of a housekeeping transcript, which is valuable for assessing preanalytic factors such as viable cellularity, collection, preservative, shipping, and storage.
b. Spiked RNA controls are mixes of multiple synthetic RNAs at a known concentration and of a known sequence that can be used to evaluate assay performance within the patient specimen.
c. Iatrogenic inhibition can be caused by the presence of residual phenol or heparin anticoagulant.
d. When multiple controls are used, the expected failure rate decreases.

Rationale: When multiple controls are used, the expected failure rate increases accordingly. For example, a failure rate of 5% for any one control implies that a combination of four controls will fail 18% of the time.

6. The phosphatidylinositol 3'-kinase (*PIK3CA*) gene encodes for the catalytic subunit of a lipid kinase that regulates signaling pathways downstream of epidermal growth factor receptor. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:56-60; DOI: 10.1016/j.jmoldx.2010.08.004; the authors of the referenced article did not disclose any relevant financial relationships.]

a. The activation of mutations in *PIK3CA* results in gain-of-function and oncogenic transformation, causing up-regulation of the AKT signaling pathway.
b. *PIK3CA* mutations may indicate a tumor that will respond to drugs targeted at genes downstream of PIK3CA in the AKT/mTOR (mammalian target of rapamycin) signaling cascade.
c. Tumors with mutations in exon 9 of *PIK3CA* are associated with poor response to cetuximab.
d. *PIK3CA* mutations have been associated with a significant increase in colon cancer-specific mortality and shorter breast cancer-specific and disease-free survival.

Rationale: Tumors with mutations in exon 20 of *PIK3CA* are associated with poor response to cetuximab.

7. *PIK3CA* is a potentially valuable marker for cancer treatment. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 13:56-60; DOI: 10.1016/j.jmoldx.2010.08.004; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Mutations in *PIK3CA* are not exclusive of mutations in *KRAS*, *BRAF*, or *NRAS*.
b. Most somatic mutations in *PIK3CA* occur in codons 542 and 545 (helical domain) and in codon 1047 (kinase domain).
c. There is a pseudogene of *PIK3CA* on chromosome 22, spanning exons 9 through 13 with >95% sequence homology, which can interfere with the detection of mutations in the helical domain exons.
d. The sequence for codons 542 and 545 are identical in the gene and the pseudogene.

Rationale: While the sequence for codon 542 is the same in the gene and the pseudogene, the pseudogene sequence corresponding to codon 545 is GCG (instead of GAG in the gene). Codons 542 and 545 are in exon 9 of the helical domain of *PIK3CA*. The authors designed a pyrosequencing assay to detect mutations in all three positions of codons 542 and 545 in exon 9 and codon 1047 in exon 20 of *PIK3CA*, and the exon 9 reverse PCR primer was designed to avoid amplifying the pseudogene, which has 95% homology with exons 9 through 13 in *PIK3CA*.

8. Human metapneumovirus (hPMV) is a leading cause of acute respiratory infection. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 14:61-64; DOI: 10.1016/j.jmoldx.2011.09.004; the authors of the referenced article did not disclose any relevant financial relationships.]

a. hPMV circulates predominantly in late fall and winter.
b. There are two distinct hPMV groups that are further divided into five subgroups A1, A2a, A2b, B1, and B2.
c. A nested PCR-restriction fragment length polymorphism assay was used to perform genotype analysis in over 4500 South Korean pediatric patients over a 3.5-year period.
d. 7.1% of the samples tested were positive for hPMV.

Rationale: hPMV circulates predominantly in late winter and spring. In some studies, respiratory syncytial virus (RSV) is a principal cause of co-infection, presumably because hPMV and RSV have similar seasonal distributions.

9. Circulating strains of hPMV differ in different populations and vary over time. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 14:61-64; DOI: 10.1016/j.jmoldx.2011.09.004; the authors of the referenced article did not disclose any relevant financial relationships.]

a. All five hPMV genotypes were detected in the patient population.
b. During the 3.5-year study period, the predominant genotype changed from A2a to B2 and back to A2a.
c. In the present study, the rate of co-infection was lower than previously reported in Korea (16% versus 25.9%).
d. One patient showed evidence of re-infection, but with a different genotype, suggesting that hPMV infection with one genotype does not confer protection against re-infection with another.

Rationale: No genotype A1 infection was detected during the study.

10. Cystic fibrosis (CF) is the most frequent lethal genetic disorder among whites. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 14:81-89; DOI: 10.1016/j.jmoldx.2011.09.001; the authors of the referenced article did not disclose any relevant financial relationships.]

a. CF phenotypic expression generally includes altered sweat test results, pancreatic insufficiency, and pulmonary infections that gradually lead to respiratory insufficiency.
b. The umbrella term of CF transmembrane conductance regulator (*CFTR*)-related disorders (*CFTR*-RDs) refers to less severe forms of CF that manifest with pancreatic insufficiency and normal or borderline sweat test results.
c. CF is caused by alterations of an ATP-dependent chloride channel, expressed by most epithelial cells.
d. Approximately 1600 mutations have been identified on the *CFTR* gene.

Rationale: Less severe forms of CF manifest with pancreatic sufficiency, normal or borderline sweat test results, and single-organ involvement. The most widely studied *CFTR*-RDs are congenital bilateral absence of the vas deferens (CBAVD), recurrent pancreatitis, and disseminated bronchiectasis.

11. Disease-causing mutations in *CFTR*-RD patients with undetected *CFTR* mutations may be located in noncoding regions of *CFTR* or in genes encoding proteins that interact with CFTR, that modulate its activity, or that display substituting channel activity. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 14:81-89; DOI: 10.1016/j.jmoldx.2011.09.001; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Large gene rearrangements are present in approximately 3% of alleles from *CFTR*-RD patients.
b. Some members of the solute carrier 26 (SLC26) family interact with CFTR protein and induce a sixfold increase in CFTR channel activity.
c. Mutations putatively responsible for CF have been identified in the amiloride-sensitive epithelial sodium channel (ENaC) in patients with CF.
d. ENaC subunits are encoded by three sodium channel, nonvoltage-gated (SCNN) genes (ie, *SCNN1A*, *SCNN1B*, and *SCNN1G*).

Rationale: Large gene rearrangements are present in approximately 3% of alleles from patients with classic CF but are absent or rare in *CFTR*-RD.

12. The molecular epidemiological features of *CFTR*-RD often include mild mutations, which are usually not included in CF mutation panels. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 14:81-89; DOI: 10.1016/j.jmoldx.2011.09.001; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Twenty-eight different mutations were identified in 99 patients affected by *CFTR*-RD.
b. The distribution of *CFTR* mutations was different within the three groups of *CFTR*-RD patients (congenital bilateral absence of the vas deferens, recurrent pancreatitis, and disseminated bronchiectasis).
c. A novel 1525-1delG *CFTR* mutation that impairs exon 10 splicing was identified in four *CFTR*-RD patients.
d. The allele frequency of 10 *SLC26A3* gene variants was significantly higher in *CFTR*-RD patients than in non-CF controls.

Rationale: A role of mutations in *SLC26A* in the pathogenesis of *CFTR*-RD was excluded. The authors identified 10 gene variants in *SLCA26A3*, of which five were novel. One variant was in the promoter, five were in introns, and four were in exons. The allele frequency of these variants did not differ significantly between *CFTR*-RD patients and controls.

ASIP 2012 Journal CME Programs

ASIP Semiannual 2012 JMD CME Program in Molecular Diagnostics

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

***The Journal of Molecular Diagnostics*, Volume 14, Number 2 (March 2012)**

www.asip.org/CME/journalCME.htm

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

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

1. Loss-of-function defects in DNA mismatch repair (MMR) occur in approximately 15% of all colorectal carcinomas (CRCs). Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:91-103; DOI: 10.1016/.jmoldx.2011.11.001; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Independent prognostic variables for CRC include stage, grade, angiolymphatic invasion, carcinoembryonic antigen level and DNA MMR status.
b. Protein heterodimers of MutS homologues (MSH2, MSH6) and of MutL homologues (MLH1, PMS2) are requisite components of the human multimeric DNA MMR protein complexes that correct strand alignment and base matching errors during DNA replication.
c. Loss-of-function defects in MMR manifest as error-prone DNA replication and microsatellite instability (MSI).
d. CRC cell lines with defective MLH1 or MSH2 show a fivefold increase in the rate of dinucleotide repeat length changes per locus per generation when compared with a MMR-proficient (pMMR) cell line.

Rationale: CRC cell lines with defective MLH1 or MSH2 show a three-log increase in the rate of dinucleotide repeat length changes per locus per generation when compared with a pMMR cell line.

2. Most deficient MMR (dMMR) CRCs are somatically acquired. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:91-103; DOI: 10.1016/.jmoldx.2011.11.001; the authors of the referenced article did not disclose any relevant financial relationships.]

a. A bimodal distribution of MSI can be observed with the use of panels of microsatellites to screen CRCs, with most cases showing <20% or >60% of microsatellites to be unstable.
b. An empirical cutoff at 40% unstable microsatellites has been adopted.
c. MSI-high (MSI-H) cases correlate with differences in stage at presentation and improved stage-specific prognosis and are considered dMMR.
d. CRCs diagnosed in the inherited Lynch (also known as hereditary nonpolyposis colorectal cancer) and Muir-Torre syndromes account for a subgroup of 2% to 3% of dMMR cases that are due to inherited/germline mutation of one allele of an MMR gene.

Rationale: An empirical cutoff at 30% unstable microsatellites has been adopted, resulting in three test results: MSI-high (MSI-H); ≥30% MSI), MSI-low (MSI-L; 0< MSI <30%), and microsatellite stability (MSS; MSI=0%).

3. Inherited and sporadic dMMR subgroups differ in origin but share a final common pathogenesis in terms of loss of MMR protein function/expression and MSI-H. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:91-103; DOI: 10.1016/.jmoldx.2011.11.001; the authors of the referenced article did not disclose any relevant financial relationships.]

a. With the exception of CRCs due to *MSH2*, MSI-low (MSI-L) cases arise and behave like microsatellite stability (MSS) CRCs.
b. In contrast to patients with pMMR CRC, MSI-H cases have improved stage-specific prognoses.
c. Patients with dMMR do not benefit from 5-fluorouracil (5-FU) chemotherapy.
d. Clinical geneticists use MMR status to scrfeen for Lynch syndrome and to counsel probands' unaffected family members.

Rationale: Except for CRCs due to *MSH6* gene mutations, MSI-L cases arise and behave like MSS cases and are considered to be pMMR.

4. Most sporadic dMMR CRCs arise in the proximal colon of older adults. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:91-103; DOI: 10.1016/.jmoldx.2011.11.001; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Most sporadic dMMR CRCs arise in sessile serrated adenomas/polyps (SSA/Ps).
b. SSA/Ps with dysplasia are considered the precursor for sporadic dMMR CRC and show unique molecular features, including *BRAF* c.1799T>A mutation, generalized decrease in CpG island methylation, MLH1 promoter hypomethylation, and MSI-H.
c. *MLH1* promoter hypermethylation (PHM) is rarely detected in MSS CRC or Lynch CRC.
d. Acquired *MLH1* PHM in Lynch syndrome can be the basis for loss of function of the remaining MLH1 wild-type allele.

Rationale: Unique molecular features of SSA/Ps with dysplasia include *BRAF* c.1799T>A mutation, *MLH1* PHM, MSI-H and the CpG island methylator phenotype (CIMP), ie generalized increase in CpG island methylation. Like its SSA/P precursor lesion, most invasive sporadic dMMR CRC exhibits MSI-H and loss of function of the MLH1 protein due to CpG island hypermethylation in the *MLH1* gene promoter.

5. The critical relevance of diagnosing patients as having Lynch syndrome relates to patient follow-up and family testing. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:91-103; DOI: 10.1016/.jmoldx.2011.11.001; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Mutations involved in Lynch syndrome include MMR gene point mutations, small germline deletions involving *MSH6*, germline deletions of the *EPCAM* gene upstream of *MSH2*, and germline *MLH1* PHM.
b. Age distribution in Lynch syndrome is unimodal with a mode at the age of 45 to 50 years and a range of 25 to 70 years.
c. Penetrance for CRC is estimated to be 80% by the age of 80 years but is dependent on the underlying mutation.
d. Approximately 50% of heritable dMMR CRC patient family members who are approached avail themselves of counseling opportunities, and 95% of those counseled undergo recommended MMR gene mutation testing.

Rationale: Mutations involved in Lynch syndrome include MMR gene point mutations, large germline deletions involving *MSH2* or *MLH1*, germline deletions of the *EPCAM* gene upstream of *MSH2*, and germline *MLH1* PHM.

6. The incomplete sensitivity of any single testing strategy for CRC emphasizes that these tests should not be used alone or even as single initial screening tests in a multitest algorithm. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:91-103; DOI: 10.1016/.jmoldx.2011.11.001; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Amsterdam II screening criteria are based on family history of Lynch-associated carcinomas and the identification of CRC in one person younger than 50 years. These criteria have a sensitivity of 42% to 50% and a specificity of 97% to 98% for the detection of associated MMR gene mutations
b. Revised Bethesda criteria are based on family history of Lynch-associated carcinoma, patient age at diagnosis, MSI-H histologic findings, and a history of other Lynch-associated carcinomas. One series using these criteria showed a sensitivity of 95% and a specificity of 38% in the detection of underlying MMR gene mutations.
c. Immunohistochemistry (IHC) for the presence of a mutation in a given MMR gene has a high interobserver κ of 0.81 to 0.93.
d. Sensitivity of MSI-H for germline mutations in MMR genes is 89% to 92% for *MLH1* mutations, 90% to 93% for *MSH2* mutations, 25% to 76% for *MSH6* mutations, and 67% for *PMS2* mutations

Rationale: Sensitivity of a mutation in a given MMR gene is 81%, 88%, and 76% for *MLH1*, *MSH2*, and *MSH6*, respectively. IHC has a mediocre to substantial interobserver κ of 0.49 to 0.79, which varies by expertise of the pathologist.

7. Placental insufficiency-related complications (PIRCs) are one of the leading causes of maternal and perinatal morbidity and mortality. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:160-167; DOI: 10.1016/j.jmoldx.2011.11.003; the authors of the referenced article did not disclose any relevant financial relationships.]

a. During the last decade there has been a trend in prenatal medicine to develop noninvasive methods to monitor an excessive placental trophoblast apoptosis associated with placental insufficiency.
b. Initially, researchers focused on the detection of male fetal-derived DNA in maternal circulation, usually using the single-copy sex-determining region Y (*SRY*) sequence on the Y chromosome, which is absent in the maternal genome.
c. The *RASSF1A* sequence is a promising universal fetal DNA marker because its promoter is hypermethylated in the fetal part of the placenta and therefore resistant to methylation-sensitive restriction enzyme digestion.
d. For differentiation between normally progressing and complicated pregnancies, the *DYS-14* sequence was an optimal marker for extracellular fetal DNA quantification.

Rationale: The *DYS-14* was not an optimal marker for differentiation between normally progressing and complicated pregnancies because of considerable variations in *DYS-14* copy numbers in males and discrepancies in *DYS-14* copy numbers between extracellular fetal DNA and the original fetal genome.

8. Many microRNAs (miRNAs) are abundantly expressed in the human placenta. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:160-167; DOI: 10.1016/j.jmoldx.2011.11.003; the authors of the referenced article did not disclose any relevant financial relationships.]

a. miRNAs belong to a family of small, noncoding RNAs that regulate gene expression at the posttranscriptional level by degrading or blocking translation of mRNA targets.
b. The absolute quantification study of seven placenta-specific miRNAs (miR-520a*, miR-520h, miR-525, miR-526a, miR-516-5p, miR-517*, and miR-518b) revealed that their levels increased in maternal circulation during progression of normally progressing pregnancies, which may be linked to the accruing mass of the placenta.
c. The levels of the seven placenta-selected miRNAs in maternal plasma showed no difference between those with normally progressing pregnancies and those with intrauterine growth restriction (IUGR), but were significantly higher at the time of preeclampsia onset.
d. Significant elevation of extracellular miRNA levels was observed during early gestation (ie, within the 12^{th} to 16^{th} weeks) in pregnancies with later onset of preeclampsia and/or IUGR, suggesting that early gestation extracellular miRNA screening can differentiate between women with normally progressing pregnancies and those who may later develop PIRCs.

Rationale: The levels of the seven placenta-selected miRNAs in maternal plasma showed no difference between those with normally progressing pregnancies and those with clinically established preeclampsia and/or IUGR.

9. Sepsis results from a host's systemic inflammatory response to infection. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 14:176-184; DOI: 10.1016/j.jmoldx.2011.12.004; the authors of the referenced article did not disclose any relevant financial relationships.]

a. The temporal separation between initial clinical suspicion of bloodstream infection and confirmation of infection often results in the early and sustained delivery of potent broad-spectrum antibiotics aimed at covering the most likely pathogens as a safe-first strategy because delay in appropriate antimicrobial therapy is associated with increased mortality.
b. Antibiotic overuse has been implicated in the emergence of drug-resistant organisms and increasing rates of *Clostridium difficile* superinfections.
c. PCR-based approaches using primers targeting the 28S ribosomal RNA (rRNA) gene can detect DNA from a wide range of bacterial pathogens with high analytical sensitivity.
d. High-resolution melting analysis (HRMA) may be a useful, low-cost method for speciation of pathogens after broad-range PCR.

Rationale: PCR-based approaches using primers targeting the 16S rRNA gene can detect DNA from a wide range of bacterial pathogens with high analytical sensitivity and can provide rule-in evidence for the presence of infection. Subsequent identification of the pathogen species is critical for focusing and de-escalation of antibiotic therapy. This can be achieved by a range of techniques, including species-specific hybridization probes, electrospray mass spectrometry, gene sequencing, and HRMA.

10. Molecular techniques aimed at the detection of circulating pathogen DNA have the potential to improve the timeliness of infection diagnosis. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 14:176-184; DOI: 10.1016/j.jmoldx.2011.12.004; the authors of the referenced article did not disclose any relevant financial relationships.]

a. The referenced study utilized Gram-positive and Gram-negative specific primers to provide an initial Gram classification of the pathogen DNA and ideal amplicon sizes for subsequent HRMA.
b. The same conditions were used for all PCRs.
c. A single-run, real-time PCR-HRMA assay, combined with a multiparameter decision-tree analysis was validated for the rapid identification of a syndromic panel of 32 organisms covering >90% of bloodstream bacterial infections encountered in the US and Europe.
d. Five bacteria were used as reference organisms for identification of the bloodstream infection panel and were routinely included in each PCR run.

Rationale: The syndromic panel included 21 organisms covering >95% of bloodstream bacterial infections encountered in the US and Europe.

11. Organisms can be grouped according to Gram typing and HRMA. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 14:176-184; DOI: 10.1016/j.jmoldx.2011.12.004; the authors of the referenced article did not disclose any relevant financial relationships.]

a. In the referenced study, organisms were allocated into one of five groups based on Tm.
b. Group 1 consisted of four Gram-positive staphyloccal species.
c. Group 2 consisted of six Gram-negative organisms.
d. Group 3 contained a mixture of Gram-positive and Gram-negative organisms.

Rationale: Organisms were allocated into one of three groups based on Tm. The differences in Tm for the three groups were highly significant (P<0.0001, analysis of variance with Gabriel's post hoc test).

12. Gram classification of unknown DNA can simplify subsequent speciation by HRMA. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2011, 14:176-184; DOI: 10.1016/j.jmoldx.2011.12.004; the authors of the referenced article did not disclose any relevant financial relationships.]

a. In many cases, differences in curve shape between species when compared with a single reference organism may be subtle.
b. The real-time PCR-HRMA technique has a laboratory analytical sensitivity of approximately 40 fg.
c. Although a precise identification of multiple organisms in a polymicrobial infection is not possible, the presence of multiple melting peaks and, in some circumstances, the result of the Gram-typing PCR are robust indicators that multiple organisms are present.
d. At present, the assay does not provide information on likely antibiotic susceptibility.

Rationale: The real-time PCR-HRMA technique has a laboratory analytical sensitivity of approximately 250 fg.

ASIP 2012 Journal CME Programs

ASIP Semiannual 2012 JMD CME Program in Molecular Diagnostics

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

The Journal of Molecular Diagnostics, Volume 14, Number 3 (May 2012)

www.asip.org/CME/journalCME.htm

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

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

1. The Prader-Willi/Angelman syndrome critical region (PWS/ASCR), located at 15q11-q13, is associated with several diseases. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:192-198; DOI: 10.1016/.jmoldx.2012.01.005; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Prader-Willi syndrome (PWS) is caused by loss of expression from genes on the paternally inherited allele at the PWS/ASCR.
b. Angelman syndrome (AS) is caused by absence of expression from the maternal allele of *UBE3A* within the PWS/ASCR locus.
c. Interstitial deletions of approximately 5 MB in length account for approximately 70% of cases of PWS and AS.
d. Imprinting errors account for 30% of cases of both PWS and AS.

Rationale: Imprinting errors, most often due to small deletions that disrupt establishment of the correct imprint during gametogenesis, account for a small percentage of cases of both PWS and AS.

2. When PWS or AS is suspected, the first-line clinical test is DNA methylation analysis of the small nuclear ribonucleoprotein polypeptide N gene (*SNRPN*). Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:192-198; DOI: 10.1016/.jmoldx.2012.01.005; the authors of the referenced article did not disclose any relevant financial relationships.]

a. *SNPRN* is an imprinted gene located within the PWS/ASCR that is associated with a differentially methylated CpG island.
b. Approximately 10% of patients with PWS have maternal uniparental disomy (UPD) for chromosome 15 (matUDP15), resulting in absence of paternal gene expression.
c. Approximately 7% of AS patients have paternal UDP15 (patUDP15), resulting in absence of gene expression of the maternally inherited *UBE3A* gene.
d. Point mutations in the maternally inherited *UBE3A* gene are found in up to 11% of AS cases.

Rationale: Approximately 25% of patients with PWS have matUDP15.

3. Interstitial duplications and triplications of the PWS/ASCR are associated with a distinct phenotype. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:192-198; DOI: 10.1016/.jmoldx.2012.01.005; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Maternally derived 15q11-q13 duplications and triplications are the most common chromosome abnormality identified in individuals with autism spectrum disorders.
b. The presence of supernumerary marker chromosome 15 introduces additional copies of the PWS/ASCR, typically two additional copies, yielding a total of four copies of this imprinted region.
c. Supernumerary chromosomes 15 account for up to 12% of all supernumerary marker chromosomes observed during karyotyping.
d. The parent of origin for copy number gains, like the parent of origin for PWS/ASCR deletions, has an effect on the phenotype, with maternally inherited copy number gains being associated with a more severe phenotype.

Rationale: Supernumerary chromosomes 15 account for approximately half of all supernumerary marker chromosomes observed during karyotyping. Their prevalence in live births is estimated to be 1:5000.

4. The methylation index (MI) is calculated as the amount of methylated DNA divided by the amount of total DNA. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:192-198; DOI: 10.1016/.jmoldx.2012.01.005; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Quantitative real-time methylation-sensitive PCR (Q-MSP) can be used to determine the parent of origin for 15q11-q13 gains by quantifying the amount of DNA methylation at *SNRPN*.
b. In unaffected individuals, the *SNRPN* promoter is methylated on the maternally inherited allele and unmethylated on the paternally inherited allele.
c. Maternally inherited PWS/ASCR gains will change the ratio of methylated:unmethylated *SNRPN* DNA from 1:1 to 2:1 (or to 3:1 for triplications).
d. Paternally inherited PWS/ASCR gains will increase the MI.

Rationale: Paternally inherited PWS/ASCR gains will change the ratio of methylated:unmethylated *SNRPN* DNA from 1:1 to 1:2 (or to 1:3 for triplications) and the MI will decrease. The MI will increase in maternally inherited PWS/ASCR gains.

5. Ovarian cancer has the highest mortality rate among gynecologic malignancies in the United States. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:214-222; DOI: 10.1016/.jmoldx.2012.01.007; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Ovarian cancer is the fifth most common cause of cancer death in women in the US.
b. Among genes that are expression-based targets, there is a class with a discontinuous or bimodal distribution of expression. For these genes, some tumors have high levels of expression and others have little to no expression, with relatively few tumors in between.
c. Sixteen probe sets encoding 8 genes were used to identify significant association between low versus high expression and survival.
d. The molecular switch state for all probe sets was combined in an additive fashion to create a single survival score for each sample; the median long-term survival for patients with a favorable score was 65 months, compared with 29 months for patients with unfavorable or indeterminate survival scores.

Rationale: Sixteen probe sets encoding 14 different genes were identified. When combined into a single sum survival score, the top survival-significant genes identify a clinically distinct molecular subtype of malignant serous ovarian cancer.

6. Genes with a strong bimodal expression pattern in ovarian cancer are candidates for translation into robust, precise clinical diagnostic and prognostic tests. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:214-222; DOI: 10.1016/.jmoldx.2012.01.007; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Four of the 14 ovarian cancer survival-significant genes encode transcription factors that synergize to regulate transcription by binding to adjacent DNA-binding elements in the context of embryonic development of the central nervous system.
b. *GREM1* has been described as prognostic in renal cell carcinoma.
c. Internal deletions in *DPP10* have been described in malignant pleural mesothelioma.
d. Among the 14 survival-significant genes identified in the study, *SOX11*, *DPP10*, and *POU3F3* were previously identified as being associated with mesenchymal development.

Rationale: Two of the genes (*SOX11* and *POU3F3*) encode transcription factors that synergize to regulate transcription by binding to adjacent DNA-binding elements in the context of embryonic development of the central nervous system.

7. Molecular understanding of the congenital muscular dystrophies (CMDs) has greatly expanded. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:233-246; DOI: 10.1016/.jmoldx.2012.01.009; the authors of the referenced article did not disclose any relevant financial relationships.]

a. A specific diagnosis of CMD can be challenging. There is a general lack of clinical awareness, muscle pathology may not yield a definitive diagnosis, and access to and expertise in immunohistochemical staining procedures are limited.
b. CMDs can be classified into four major groups, based on the affected genes and the location of their expressed protein.
c. All four groups of CMD have a classic autosomal recessive inheritance pattern.
d. Twelve genes associated with CMDs span a 65-kb exonic region.

Rationale: Inheritance patterns range from classic autosomal recessive to *de novo* dominantly acting mutations.

8. Next-generation sequencing (NGS) applications are beginning to have an impact on molecular medicine. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:233-246; DOI: 10.1016/.jmoldx.2012.01.009; the authors of the referenced article did not disclose any relevant financial relationships.]

a. The analytical sensitivity and specificity of solution-based hybridization and microdroplet-based PCR target enrichment in conjunction with NGS were assessed to identify mutations in genes involved with CMDs.
b. In microdroplet-based PCR target enrichment, emulsion chemistry generates millions of microdroplet-based PCR products, each representing a single amplification of a desired target locus.
c. The microdroplet-based PCR target enrichment was deemed to be more appropriate for a clinical laboratory, due to excellent sequence specificity and uniformity, lower cost, high reproducibility, high coverage of the target exons, and the ability to distinguish the active gene from known pseudogenes.
d. As opposed to solution-based hybridization enrichment, the microdroplet-based PCR technology enriched all 321 exons representing the 12 genes involved with CMDs.

Rationale: Regardless of the method (solution-based hybridization or microdroplet-based PCR), exons with highly repetitive and high GC regions are not well enriched and require Sanger sequencing for completeness.

9. Extrahepatic cholangiocarcinoma (EHC) is usually difficult to diagnose by bile cytology because of cellular disintegration. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:256-263; DOI: 10.1016/j.jmoldx.2012.01.014; the authors of the referenced article did not disclose any relevant financial relationships.]

a. EHC comprises >3.0% of invasive cancers in the US.
b. Patients with EHC have a poor prognosis due to its early direct extension to adjacent tissues and organs and high metastasis rate.
c. Imaging modalities that are used to diagnose EHC include endoscopic retrograde cholangiopancreatography, endoscopic ultrasonography, and magnetic resonance imaging.
d. Cytological examinations have low sensitivity for detecting EHC, often because of a low cell count, cell disintegration, or diagnostic difficulty in differentiating reactive biliary epithelial atypia associated with inflammatory stricture from cancer cells.

Rationale: Adenocarcinoma of the extrahepatic bile duct is relatively rare, comprising <0.2% of invasive cancers in the US.

10. DNA methylation markers are promising candidates for tumor biomarkers. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:256-263; DOI: 10.1016/j.jmoldx.2012.01.014; the authors of the referenced article did not disclose any relevant financial relationships.]

a. To date, >30 genes have been reported to be hypermethylated in EHC in a cancer-specific manner, some with methylation frequencies of >70% in EHC.
b. Bile cytology will no longer be necessary in the diagnosis of EHC.
c. A five-gene panel (*CCND2*, *CDH13*, *GRIN2B*, *RUNX3*, and *TWIST1*) detected EHC at a sensitivity of 83%, which is far higher than that of bile cytology (46%, P = 0.004).
d. To increase the detection sensitivity of DNA methylation analysis of bile specimens, brushing of the bile duct epithelia in obstructed areas may increase the amount of tumor-derived DNA per unit volume of collected bile material.

Rationale: The marker panel assay will not replace bile cytology in the diagnosis of EHC because tissue confirmation of cytological diagnosis of the neoplasm is desirable for management of patients with tumors that cause strictures of the biliary duct.

ASIP Semiannual 2012/2 JMD CME Program in Molecular Diagnostics

[July, September, November issues of JMD Volume 14]

CME Accreditation Statement: This activity ("ASIP Semiannual 2012/2 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 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 ("ASIP Semiannual 2012/2 JMD CME Program in Molecular Diagnostics") for a maximum of 24 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

CONTINUING MEDICAL EDUCATION (CME) INFORMATION

CME Accreditation Statement: This activity ("ASIP Semiannual 2012/2 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 for Clinical Pathology (ASCP) and the American Society for Investigative Pathology (ASIP). ASCP is accredited by the ACCME to provide continuing medical education for physicians.

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The ASIP Semiannual 2012/2 JMD CME Program in Molecular Diagnostics is a semiannual program consisting of a series of at least 24 questions based on selected articles in the July – November 2012 issues (Volume 14, Issues 4-6) 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 a monthly exam and complete a Post-Test Evaluation. All exams must be completed by December 31, 2012 to receive CME credit. Participants will earn 8 *AMA PRA Category 1 Credit(s)*™ for successful completion of each exam.

Should you have questions, contact the ASIP Education Office (301-634-7440; email journalCME@asip.org), 9650 Rockville Pike, Bethesda, MD 20814.

SAM Credit
The ASIP Semiannual 201/2 JMD CME Program in Molecular Diagnostics is approved by the American Board of Pathology for up to 24 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 SAM applications online on the ASIP website (www.asip.org). All SAM applications must be completed by December 31, 2012 for participants to receive SAM credit.

Should you have questions about SAM credits, contact the ASIP Education Office (301-634-7440; email journalCME@asip.org), 9650 Rockville Pike, Bethesda, MD 20814.

Meeting Objective/Target Audience
The objective of the ASIP Semiannual 2012/2 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 ASIP Semiannual 2012/2 JMD CME Program in Molecular Diagnostics is designed to meet the participants' education needs in the physician competency area of Medical Knowledge, as defined by the Accreditation Council for Graduate Medical Education (ACGME) and the American Board of Medical Specialties (ABMS), and to support participants' lifelong learning towards a goal of promoting patient safety and

improving patient care and is specifically targeted to trainees, clinicians and researchers investigating mechanisms of disease who wish to advance their knowledge of the cellular and molecular biology of disease.

Educational Objectives
At the completion of the ASIP Semiannual 2012/2 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 to 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 to subsequent CME planning decisions. The primary purpose of this journal-based CME activity is educational and the comments, opinions, and/or recommendations expressed by the faculty or authors are their own and not those of ASCP or ASIP.

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

ASIP 2012 Journal CME Programs

ASIP Semiannual 2012/2 JMD CME Program in Molecular Diagnostics

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

The Journal of Molecular Diagnostics, Volume 14, Number 4 (July 2012)

www.asip.org/CME/journalCME.htm

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

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

1. Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:305-313; DOI: 10.1016/.jmoldx.2012.02.007; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. Approximately 1 in 2,500 individuals in the United States and 50 million patients worldwide are affected by ADPKD.
b. ADPKD is characterized by bilateral kidney cyst development and progressive chronic kidney disease.
c. Approximately 75% to 85% of ADPKD cases are caused by *PKD1* mutations.
d. Approximately 15% to 25% of ADPKD cases are caused by *PKD2* mutations.

Rationale: ADPKD affects approximately 1 in 500 individuals in the US and 12.5 million patients worldwide.

2. Although genetic testing of *PKD1* and *PKD2* is useful for the diagnosis and prognosis of ADPKD, analysis is complicated by several factors. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:305-313; DOI: 10.1016/.jmoldx.2012.02.007; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. *PKD1* is a large gene composed of 46 exons.
b. Humans possess at least six *PKD1* homologues, each encompassing a duplicated region from exon 1 to exon 36 on chromosome 18.
c. Until recently, the duplicated *PKD1* region has required nested PCR of each individual exon to distinguish the true gene from the highly identical homologous genes.
d. *PKD1* is highly polymorphic, with an average of 10 variants per individual.

Rationale: Pseudogenes are a particular problem in the molecular diagnosis of ADPKD. The known homologues of *PKD1* encompass a duplicated region of 33 exons on chromosome 16. The single-copy region of *PKD1* is from exon 34 to exon 46.

3. MicroRNAs (miRNAs) are highly conserved small noncoding RNAs that down-regulate gene expression. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:314-321; DOI: 10.1016/.jmoldx.2012.02.008; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. miRNAs are transcribed as large RNA precursor molecules with hairpin structures that undergo a stepwise maturation process in the nucleus and cytoplasm, ultimately becoming 18 to 25 nucleotides in length.
b. miRNAs down-regulate gene expression by translational repression or mRNA degradation.
c. The seed region of the mature miRNA binds to its complementary region in the 3' untranslated region of the target mRNA.
d. The seed region is located between nucleotides 11 and 18 of the mature miRNA.

Rationale: The seed region is located between nucleotides 2 and 8 of the mature miRNA. miRNAs are considered to be promiscuous in that each seed region may pair with multiple mRNA targets; the fidelity of pairing affects posttranscriptional regulation.

4. miRNAs have essential cellular functions. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:314-321; DOI: 10.1016/.jmoldx.2012.02.008; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. To date >1,000 human miRNAs have been identified.
b. It is estimated that >90% of human genes are regulated by miRNAs.
c. miRNAs are involved in the regulation of critical physiological processes such as embryonic development, cell survival and cycle, cell differentiation, apoptosis, and immunity.
d. miRNAs are involved in a variety of human diseases including viral infection, cancer development and aggressiveness, and immune and cardiovascular diseases.

Rationale: It is estimated that >50% of human genes are regulated by miRNAs.

5. Relative quantification compares the relative amount of a target sequence in a sample of interest with the same sequence in a sample of reference or a calibrator sample, posing several limitations for clinical applications that can be circumvented by absolute quantification approaches. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:314-321; DOI: 10.1016/.jmoldx.2012.02.008; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. In a relative quantification scheme, the calibrator sample should be available in large enough quantities to be included in each run; however, this may prohibit its use in routine procedures or long-term patient follow-up.
b. Absolute quantification using plasmid calibrators circumvents limitations of relative quantification because the initial copy number of the target is determined by relating the PCR measurement to a standard curve.
c. Plasmids are unstable at room temperature after even short storage times, posing a major limitation of plasmids as qPCR calibrators.
d. In the referenced article, generation of plasmid calibrators for absolute qPCR quantification involved cloning a synthetic molecule, avoiding PCR amplification of short miRNAs of high sequence homology within a family of miRNAs.

Rationale: Plasmids are highly stable molecules even after long storage times at room temperature. They generate reproducible standard curves with reliable efficacy and sensitivity and can be distributed around the world, expediting the comparison of results between laboratories.

6. Influenza is a widespread pathogen. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:328-335; DOI: 10.1016/.jmoldx.2012.02.001; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. Influenza results in approximately 36,000 deaths annually in the US.
b. Influenza subtypes are classified by the antigenic subtype of hemagglutinin (H1 to H16) and neuraminidase (N1 to N9) proteins expressed on the viral particles.
c. Seasonal influenza is generally caused by H3N2, H5N1, and H5N12 viruses.
d. A novel, swine-derived recombinant variant of H1N1 virus resulted in a limited pandemic in 2009.

Rationale: Seasonal influenza is generally caused by H1N1, H3N2, and H1N2 viruses. The H5N1 subtype causes the highly pathogenic avian flu.

7. A recurrent pandemic of influenza could result in millions of deaths worldwide. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:328-335; DOI: 10.1016/.jmoldx.2012.02.001; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. Influenza pandemics could be avoided if rapid point-of-care diagnostics were available to health workers at the source of the infection, thus containing the infection before it spreads to the global community.
b. Rapid diagnosis of influenza could prevent excessive antibiotic use, which leads to antibiotic-resistant bacterial strains.
c. Immunospecific tests, such as rapid antigen tests and immunofluorescence microscopy, are highly sensitive and provide sequence-specific information for subtyping influenza.
d. Although specific, viral culture requires 3 to 14 days for results, limiting its use for rapid diagnostics.

Rationale: Immunospecific tests lack sensitivity and do not provide sequence-specific information for subtyping.

8. Nucleic acid sequence–based amplification (NASBA) is faster than PCR for RNA amplification but has some limitations. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:328-335; DOI: 10.1016/.jmoldx.2012.02.001; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. NASBA is limited by RNA secondary structure that makes primer design and multiplexing difficult.
b. NASBA begins with a 70°C heating step before the addition of enzymes to disrupt full-length RNA secondary structure.
c. Poor hybridization of ssDNA oligonucleotides complicates primer design, making it difficult for many conserved regions to be successful as NASBA priming sties.
d. Primer target sites for NASBA reactions are typically 100 to 250 nucleotides apart to avoid products with inhibitory secondary structure.

Rationale: NASBA begins with a 65°C heating step before the addition of enzymes to disrupt full-length RNA secondary structure.

9. Diagnostic NASBA assays integrated with microfluidic systems show improvements over conventional benchtop techniques. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:328-335; DOI: 10.1016/.jmoldx.2012.02.001; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. The small reaction volumes inherent in microfluidic chips concentrate the target molecule of interest to improve primer binding and reaction kinetics.
b. The authors of the referenced article propose a novel “simple method for amplifying RNA targets” (SMART) that incorporates the advantages of microfulidics and avoids the limitations of NASBA.
c. SMART uses a polydimethylsiloxane (PDMS) chamber reactor.
d. The amplification step in SMART directly amplifies the starting RNA, using an engineered ssDNA probe that reduces secondary structure.

Rationale: SMART incorporates a binding step and a microfluidic separation step that allows the two hybridization sites to be located anywhere along the RNA target. Incorporation of an ssDNA probe allows the user to choose amplifiable probe and primer sequences that reduce secondary structure and enable the user to optimize reaction kinetics. However, it is important to note that the amplification step in SMART does not directly amplify the starting RNA. Instead, amplifiable probe-target RNA complexes are captured on magnetic beads using a sequence-specific capture probe and are separated from unbound probe using microfluidics.

10. Renal cell carcinoma (RCC) is the most common kidney tumor. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:385-392; DOI: 10.1016/.jmoldx.2012.02.003; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. RCC has shown a 40% decrease in incidence and a 60% decrease in mortality during the past 20 years.
b. RCC encompasses a heterogeneous group of cancers, each with distinct morphological characteristics and associated cytogenetic changes.
c. The clear cell subtype (ccRCC) accounts for approximately 75% to 80% of RCC cases.
d. Oncocytoma is a benign neoplasm of the kidney that shares genomic and morphological similarities with chromophobe RCC (chRCC).

Rationale: RCC has shown an increase in incidence and mortality during the past 20 years, despite earlier detection and introduction of new therapies.

11. Accurate classification of RCC is important because the subtypes differ in their prognosis. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:385-392; DOI: 10.1016/.jmoldx.2012.02.003; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. Distinguishing the eosinophilic variant of ccRCC from either chRCC or oncocytoma can be challenging.
b. The anatomical extent, or stage, of disease is the most useful prognostic factor for patients with RCC.
c. Lymph node involvement is the most commonly used prognostic factor for patients with metastatic disease.
d. Hybrid tumors have more than one subtype in the same tumor.

Rationale: The most commonly used prognostic models for patients with metastatic disease are based on clinical parameters.

12. An aberrant pattern of miRNA expression has been observed in RCC. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:385-392; DOI: 10.1016/.jmoldx.2012.02.003; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. miR-21 expression is significantly lower in the clear cell and papillary subtypes of RCC compared with chRCC, oncocytoma, and healthy kidney tissues.
b. Expression levels of miR-21 are comparable in both chRCC and oncocytoma.
c. Kaplan-Meier survival curves show a clear demarcation between miR-21–positive and mir-21–negative patients.
d. Predicted miR-21 targets that are key molecules in RCC pathogenesis include *VHL*, *EGLN1*, *PTEN*, *TSC1*, and *TSC2*.

Rationale: miR-21 expression is significantly higher in the clear cell and papillary subtypes of RCC compared with chRCC, oncocytoma, and healthy kidney tissues. Kaplan-Meier survival curves demonstrate a significantly higher disease-free survival and overall survival in miR-21–negative patients compared to miR-21–positive patients.

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

1. Sudden cardiac death (SCD) due to ventricular arrhythmia most commonly occurs in the setting of coronary artery disease. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:424-436; DOI: 10.1016/.jmoldx.2012.04.002; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. Approximately 1,000 people experience SCD each year in the United States.
b. Familial cardiac syndromes associated with SCD may be divided into those associated with structural heart disease and the primary electrical disorders with structurally normal hearts.
c. SCD syndromes associated with structural heart disease include hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC).
d. SCD syndromes associated with the primary electrical disorders with structurally normal hearts include long QT syndrome (LQTS), short QT syndrome (SQTS), Brugada syndrome (BrS), and catecholaminergic polymorphic ventricular tachycardia (CPVT).

Rationale: Approximately 350,000 people experience SCD each year in the United States.

2. HCM is an autosomal dominant disorder marked by unexplained and often asymmetric hypertrophy of the left ventricle (LV). Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:424-436; DOI: 10.1016/.jmoldx.2012.04.002; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. Women are affected more commonly by HCM than men (ratio of 3:2).
b. With a prevalence of approximately 1 in 500, HCM is the most common inherited cardiac disease.
c. Symptoms of HCM may include shortness of breath on exertion, syncope, or SCD related to ventricular arrhythmia.
d. HCM is diagnosed by the presence of LV hypertrophy without an alternative cause.

Rationale: Men are affected more commonly by HCM than women (ratio of 3:2).

3. ARVC is characterized pathologically by fibrofatty replacement of the right ventricular myocardium and genetically as a disease of the desmosome. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:424-436; DOI: 10.1016/.jmoldx.2012.04.002; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. Males are more commonly diagnosed as having ARVC than females (ratio of 3:1).
b. The estimated prevalence of ARVC is 1 in 5,000, but some areas in Italy have a lower prevalence (4 per 100,000).
c. The first identified genetic cause of ARVC was a homozygous mutation in plakoglobin (*JUP*), causing Naxos syndrome.
d. Treatment of ARVC involves restriction of high-intensity exercise and the implantation of an implantable cardioverter defibrillator (ICD) in the event of ventricular arrhythmia.

Rationale: The estimated prevalence of ARVC is 1 in 5,000, but the prevalence in some areas in Italy is as high as 4.4 per 1,000.

4. Congenital LQTS is marked by a prolonged QT interval, torsades de pointes, and a risk of SCD. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:424-436; DOI: 10.1016/.jmoldx.2012.04.002; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. LQTS is more commonly diagnosed in females than in males (ratio of 2:1).
b. LQTS is usually identified in the investigation of syncope in a young person and sometimes incidentally on an electrocardiogram (ECG) performed for other reasons.
c. At present, mutations in 5 genes have been implicated in autosomal dominant LQTS.
d. Beta-adrenergic blockade is indicated for most patients with this condition, with an ICD reserved for those at very high risk or with breakthrough cardiac events while taking medication.

Rationale: At present, mutations in >10 genes have been implicated in autosomal dominant LQTS and are often referred to by their subtype number, reflecting the order of their discovery.

5. *Mycoplasma* and *Ureaplasma* species are well-known pathogens responsible for a broad array of inflammatory conditions involving the respiratory and urogenital tracts of neonates, children, and adults. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:437-450; DOI: 10.1016/.jmoldx.2012.06.001; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. Mycoplasmas are included within the phylum Tenericutes, class Mollicutes.
b. Mollicutes are smaller than conventional bacteria, in cellular dimensions and genome size, making them the smallest free-living organisms known.
c. Lack of a cell wall, extremely small genome, and limited biosynthetic capabilities explain the parasitic or saprophytic existence of these organisms, their sensitivity to environmental conditions, resistance to β-lactam antibiotics, and fastidious growth requirements.
d. Among the mollicutes, genome sizes range from 580 to 2,200 kbp, with *Mycoplasma genitalium* being among the largest.

Rationale: Among the mollicutes, genome sizes range from 580 to 2,200 kbp, with *M. genitalium* being the smallest.

6. *M. pneumoniae* is a common cause of upper and lower respiratory tract infections in children and adults. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:437-450; DOI: 10.1016/.jmoldx.2012.06.001; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. *M. pneumoniae* is easily spread through respiratory droplets and can cause a variety of clinical manifestations, including pharyngitis, tracheobronchitis, and pneumonia.
b. Extrapulmonary manifestations sometimes occur after primary respiratory tract infection, either by direct spread or autoimmune effects.
c. Attachment of *M. pneumoniae* to host cells in the respiratory tract is not required for colonization and infection.
d. *M. pneumoniae* stimulates B and T lymphocytes and induces formation of autoantibodies that react with a variety of host tissues and the I antigen on erythrocytes, which is responsible for production of cold agglutinins.

Rationale: Attachment of *M. pneumoniae* to host cells in the respiratory tract is required for colonization and infection.

7. Serological testing was the first method developed for detection of *M. pneumoniae* infections. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:437-450; DOI: 10.1016/.jmoldx.2012.06.001; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. The main disadvantages of serological analysis are the requirement for acute and convalescent serum samples that are tested simultaneously for IgM and IgG to confirm seroconversion, difficulty in distinguishing current or recent infection from past infection, and need to wait 1 to 2 weeks from onset of the infection until detectable antibody develops.
b. More recently, molecular-based nucleic acid amplification tests (NAATs) have reduced the need for serological diagnosis.
c. NAATs are also useful for the identification of organisms grown in culture to the species level, replacing older and cumbersome technologies.
d. PCR is the least used NAAT for detection of *Mycoplasma* and *Ureaplasma* species.

Rationale: PCR is the most widely applied NAAT for detection of *Mycoplasma* and *Ureaplasma* species.

8. Members of the genus *Ureaplasma* hydrolyze urea and use it as a metabolic substrate for generation of ATP. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:437-450; DOI: 10.1016/.jmoldx.2012.06.001; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. There are seven recognized *Ureaplasma* species, with *U. parvum* and *U. urealyticum* being the two species found in humans.
b. Although detected less frequently than *U. urealyticum* in most patient populations, *U. parvum* may be more pathogenic in male urethritis.
c. As many as 40% to 80% of healthy adult women may harbor *ureaplasmas* in their cervix or vagina.
d. *Ureaplasma* species reside primarily on the mucosal surfaces of the urogenital tracts of adults or the respiratory tracts in infants.

Rationale: Although detected less frequently than *U. parvum* in most patient populations, *U. urealyticum* may be more pathogenic in male urethritis.

9. Genetic testing and research have increased the demand for high-quality DNA that has traditionally been obtained by venipuncture. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:451-457; DOI: 10.1016/.jmoldx.2012.04.005; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. Over the years, the process of tissue selection has been driven by convention, convenience, or a history of sample performance from high-profile scientific publications.
b. Venous blood collection is the easiest and most practical method for international collaborative investigation with large-scale specimen collection or exchange.
c. Guthrie/FTA card–based blood spots, buccal scrapes, and finger nail clippings are DNA-containing specimens that are uniquely accessible and thus attractive as alternative tissue sources (ATS).
d. The ease of collection, storage, and lower cost of sample shipment and extraction make ATS a valuable resource to stand alone or complement other specimens that may be limited in quality, scope, or yield.

Rationale: Venous blood collection may prove difficult in special populations and when large-scale specimen collection or exchange is prerequisite for international collaborative investigations.

10. The BK virus is one of three polyomaviruses (BK, JC, and Merkel) in the human polyomavirus family. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:494-500; DOI: 10.1016/.jmoldx.2012.04.004; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. The human BK virus is a relatively small (5.1 kb), common enveloped double-stranded DNA virus.
b. BK viral nephropathy (BKVN) is an emerging cause of kidney transplant failure, affecting 1% to 10% of patients and leading to graft loss in 15% to 80% of cases within 5 years.
c. BKVN progresses through stages, including an initial cytopathic stage followed by a cytopathic-inflammatory stage, and finally a late stage characterized by tubular atrophy and fibrosis.
d. The lytic destruction of BK-infected tubular renal epithelium induces an influx of activated T lymphocytes similar to the interstitial infiltrate of T cells associated with acute allograft rejection.

Rationale: The human BK virus is a relatively small (5.1 kb), common nonenveloped double-stranded DNA virus.

11. BKVN is the most frequent infectious complication after kidney transplantation. Based on the referenced article, select the ONE statement that is NOT true: See J Mol Diagn 2012, 14:494-500; DOI: 10.1016/.jmoldx.2012.04.004; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. In most patients who have received a renal transplant, BK viral load monitoring is performed by plasma or urine real-time PCR (qPCR).
b. Detection of BK virus via qPCR directly correlates to active renal infection of BKVN.
c. Histologic detection of BK virus in renal biopsy specimens depends on H&E-based identification of viral inclusions or immunohistochemistry (IHC).
d. BK viral inclusions within tubular epithelia can be recognized via conventional H&E staining by identification of one of four different structural variants: amorphous basophilic ground-glass variant, eosinophilic-granular halo-encircled inclusion, finely granular type lacking a halo, and vesicular variant with markedly enlarged nuclei and clumped irregular chromatin.

Rationale: Detection of BK virus via qPCR does not directly correlate to active renal infection of BKVN.

12. Although viral inclusions are characteristic on H&E, they are sometimes missed as a consequence of sampling variability, atypical appearance, or variable distribution within tissue. Based on the referenced article, select the ONE statement that is NOT true: See J Mol Diagn 2012, 14:494-500; DOI: 10.1016/.jmoldx.2012.04.004; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. The most common structural BK viral inclusion is amorphous basophilic ground-glass variant.
b. IHC-based detection has a higher sensitivity than H&E based detection; however, antibody-mediated detection uses antibody with specificity for simian virus 40 (SV40) and is therefore not specific for the BK virus.
c. Formalin fixation and paraffin embedding can alter epitope–antibody interaction and decrease sensitivity of IHC-based detection.
d. The identification of BK virus via *in situ* hybridization is a routinely used BK virus specific detection approach in clinical laboratories.

Rationale: An alternate BK virus specific detection approach is *in situ* hybridization; however, this approach is not currently used.

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

1. Fragile X syndrome (FXS) is the most common form of X-linked inherited intellectual disability. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:560-568; DOI: 10.1016/.jmoldx.2012.05.003; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. FXS has an estimated prevalence of 1 in 7000 to 8000 males and 1 in 4000 females.
b. FXS is associated with moderate to severe intellectual and social impairment, anxiety, attention-deficit/hyperactivity disorder, and autism.
c. The phenotypic presentation is variable but characteristic physical features include a prominent forehead, a long narrow face, protruding ears, and macroorchidism in adolescent and adult males.
d. 50% to 60% of affected females develop mild to moderate intellectual disability.

Rationale: FXS has an estimated prevalence of 1 in 4000 males and 1 in 7000 to 8000 females.

2. Molecularly, FXS is almost exclusively characterized by an expansion of a $(CGG)_n$ trinucleotide repeat, located in the 5'-untranslated region of the fragile X mental retardation 1 (*FMR1*) gene encoding the FMRP RNA-binding protein. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:560-568; DOI: 10.1016/.jmoldx.2012.05.003; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. The frequency of premutation carriers in the general population varies between 1 in 251 and 1 in 813 males and between 1 in 113 and 1 in 259 females.
b. The normal form of the CGG repeat is highly polymorphic in the general population and contains 6 to approximately 50 trinucleotide repeats, with alleles containing 29 to 30 trinucleotide repeats being the most prevalent.
c. Intermediate trinucleotide lengths range between 60 and 75 repeats and are slightly unstable on transmission from generation to generation but rarely jump to full expansions over a single meiosis.
d. Many *FMR1* alleles contain AGG sequences that are interspersed among the CGG triplets and are believed to confer stability and to reduce the risk of expansion.

Rationale: Intermediate trinucleotide lengths range between 51 and 58 repeats and are slightly unstable on transmission from generation to generation but rarely jump to full expansions over a single meiosis.

3. Molecular diagnosis of FXS relies on accurate and efficient determination of the number of the triplet repeat elements in the promoter region of *FMR1*. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:560-568; DOI: 10.1016/.jmoldx.2012.05.003; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. Routine molecular analysis includes PCR amplification with primers flanking the CGG repeat.
b. Currently, Southern blot analysis of alleles is required to detect larger alleles not amplified by PCR and to resolve zygosity in female samples in which there may be any ambiguity about a missed full-mutation (FM) allele.
c. Currently, nearly 25% of all females in the general population must be processed by Southern blot analysis.
d. Recent advances in methylation PCR protocols may allow determination of the full set of information without Southern blot analysis in the near future.

Rationale: More than a third of all females in the general population must be processed by Southern blot analysis for FXS. Standard fragile X PCR analysis is not designed to discriminate heterozygotes with alleles one repeat apart from homozygotes or from heterozygotes with a missed large expansion (apparent homozygosity).

4. Alport's syndrome (AS) is clinically heterogeneous hereditary nephritis. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:586-593; DOI: 10.1016/.jmoldx.2012.06.005; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. AS is characterized by hematuria, progressive renal failure, hearing loss, and ocular lesions.
b. Leiomyomatosis occurs in some AS patients.
c. Diffuse thickening of the glomerular basement membrane with splitting of the lamina densa is the characteristic ultrastructural change of AS.
d. About 58% of AS is X-linked (XLAS) due to mutations in the *COL4A5* gene, encoding type IV collagen α5 chain.

Rationale: About 85% of AS is XLAS due to mutations in the *COL4A5* gene, encoding type IV collagen α5 chain.

5. More than 600 mutations in *COL4A5* have been reported. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:586-593; DOI: 10.1016/.jmoldx.2012.06.005; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. There is a strong relationship between *COL4A5* mutation categories, age at onset of end-stage renal disease, and extrarenal manifestations in male patients in Europe and the United States.
b. The size and high allelic heterogeneity of the *COL4A5* gene hamper the utility of genetic analysis as a routine test for XLAS diagnosis.
c. Immunohistochemical analysis of the α5(IV) chain in the epidermal basement membrane is a useful approach for XLAS diagnosis.
d. Previously, immunological analysis demonstrated that approximately 45% of XLAS patients exhibit normal staining of α5(IV) chain in epidermal basement membrane (EBM).

Rationale: The sensitivity of immunological analysis as observed previously in a small series of AS patients is 75%, with approximately 15% of XLAS patients exhibiting normal staining of α5(IV) chain in EBM.

6. In addition to the well-established mutations in the *FLT3*, *NPM1,* and *CEBPA* genes as prognostic markers, various novel molecular abnormalities have been found in acute myeloid leukemia (AML), especially in patients with normal karyotype. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:594-601; DOI: 10.1016/.jmoldx.2012.06.006; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. Mutations in the *ASXL1* gene (6% to 30%), *IDH1* and *IDH2* genes (8% to 33%), and c-*CBL* proto-oncogene (~1%) have been found in AML patients.
b. *ASXL1* mutations seem to lead to epigenetic dysregulation.
c. *IDH* mutations result in the generation of the aberrant metabolite 2-hydroxyglutarate, which induces DNA hypermethylation and impairs differentiation in hematopoietic cells through inhibition of *TET2*.
d. *IDH2* single-nucleotide polymorphism rs11554137 may alter IDH2 activity by alterations in RNA.

Rationale: *IDH1* single-nucleotide polymorphism rs11554137 may alter IDH1 activity by alterations in RNA.

7. High-resolution melting (HRM) is a novel method to analyze genetic variations based on their sequence, length, GC content, or strand complementarity. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:594-601; DOI: 10.1016/.jmoldx.2012.06.006; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. HRM analysis demonstrated that *IDH1* and *IDH2* mutations were frequently found in the same AML patient in the studied patient cohort.
b. Patients with *ASXL1* mutations did not harbor *IDH1*, *FLT3*, or *CEPBA* mutations.
c. *NPM1* mutations were concurrently found with *ASXL1*, *IDH*1, or *IDH2* with a variable incidence.
d. Mutations were not significantly correlated with any of the clinical and biological features studied.

Rationale: *IDH1* and *IDH2* mutations were mutually exclusive. This finding is contradictory to results of a 2010 report by Paschka et al: J Clin Oncol 20120, 28:3636-3643.

8. Breast carcinogenesis is a multistep process that involves both genetic and epigenetic alterations. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:613-622; DOI: 10.1016/.jmoldx.2012.07.001; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. Identification of aberrantly methylated genes in breast tumors and their relation to clinical parameters can contribute to improved diagnostic, prognostic, and therapeutic decision making.
b. Breast cancer is a heterogeneous disease with varied histopathology, clinical behavior, prognosis, and response to treatment.
c. Invasive lobular carcinoma (ILC) comprises approximately 75% to 85% of primary breast cancers and exhibits a distinct biological behavior compared with invasive ductal carcinoma (IDC), the second most common overall breast cancer type.
d. The most studied epigenetic alteration in human neoplasms is the hypermethylation of CpG islands in gene promoter regions.

Rationale: IDC comprises approximately 75% to 85% of primary breast cancers and exhibits a distinct biological behavior compared with ILC, the second most common overall breast cancer type.

9. In the assessment of breast cancer, lymph node status, primary tumor size, and histological grade are the more informative prognostic factors. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:613-622; DOI: 10.1016/.jmoldx.2012.07.001; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. In Western countries, the estimated 5-year disease-free survival in patients with unaffected lymph nodes is approximately 80%.
b. In Argentina, patients with affected lymph nodes experience five times reduced overall survival rates and six times higher recurrence risk.
c. If lymph nodes do not contain metastasis, the most important prognostic factor is tumor size because disease recurrence rate increases as primary tumor size increases.
d. The histological characteristics of tumors can be evaluated, graded, and related to prognosis of the disease.

Rationale: In Argentina, patients with affected lymph nodes experience five times reduced overall survival rates and almost three times higher recurrence risk.

10. The histological characteristics of tumors can be evaluated, graded, and related to prognosis of the disease. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2012, 14:613-622; DOI: 10.1016/.jmoldx.2012.07.001; the authors of the referenced article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.]

a. The Nottingham combined histological grade takes into account differentiation (tubule formation), nuclear pleomorphism (nucleus/cytoplasm relation), and mitotic count (mitoses per high-power field).
b. The combined Nottingham evaluation leads to two grades: low and high.
c. Several studies have associated increased histological grade with poor prognosis.
d. During the last decade, the classification of patients with breast cancer by the traditional characteristics has been modified to include use of gene expression signatures, classifying breast tumors into six groups: normal breast-like, luminal A, luminal B, HER2-enriched, claudin-low, and basal-like.

Rationale: The combined Nottingham evaluation leads to three grades: low, intermediate, and high.