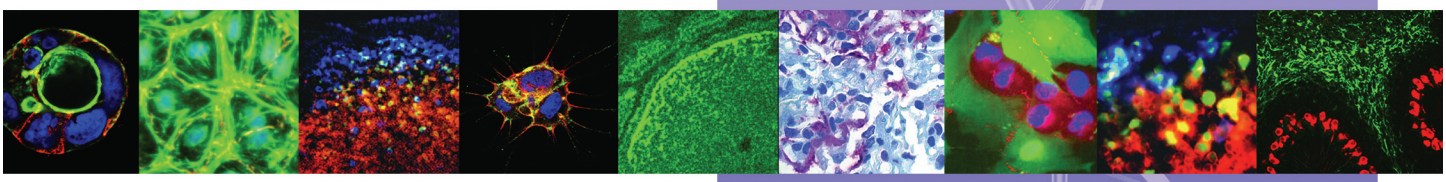
ASIP Journal CME Program

JMD CME Program in Molecular Diagnostics

Questions & Answers 2009



For more information on how to participate in the 2010 ASIP Journal CME Program see inside or visit:

www.asip.org/CME/journalCME.htm

Program Director: Mark E. Sobel, MD, PhD

ASIP 2009 Journal CME Program: JMD 2009 CME Program in Molecular Diagnostics
***The Journal of Molecular Diagnostics* (*JMD*)**
Volume 11
http://jmd.amjpathol.org
www.asip.org/CME/journalCME.htm

Mark E. Sobel, MD, PhD, Director of Journal CME Programs
Lisa McFadden, Educational Services Manager

Accreditation statement: This activity ("2009 ASIP Journal CME Program") 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 educational activity ("2009 ASIP Journal CME Program") for a maximum of 100 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

The ASIP Journal CME Program consists of two components, the AJP CME Program in Pathogenesis and the JMD CME Program in Molecular Diagnostics. Participants can elect to participate in each program component individually or to participate in both components simultaneously. FASEB designates each of the two components of this educational activity ("AJP CME Program in Pathogenesis" and "JMD CME Program in Molecular Diagnostics") for a maximum of 50 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

The JMD CME Program in Molecular Diagnostics consists of a series of 50 questions based on selected articles in the 2009 issues (Volume 11) of *The Journal of Molecular Diagnostics* (JMD). Up to 10 questions that are based on articles appearing in each bimonthly issue of JMD will be available online for registered participants. To receive credit for this journal-based CME activity, participants must answer questions based on selected articles in Volume 11 of JMD (calendar year 2009) and achieve a cumulative score of at least 75% (correct answers to at least 38 of the 50 questions in the annual program) and they must complete an Evaluation Form upon completion of the annual program.

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.

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2009 ASIP Journal CME Program:
JMD 2009 CME Program in Molecular Diagnostics

American Society for Investigative Pathology

The Journal of Molecular Diagnostics

Volume 11

http://jmd.amjpathol.org
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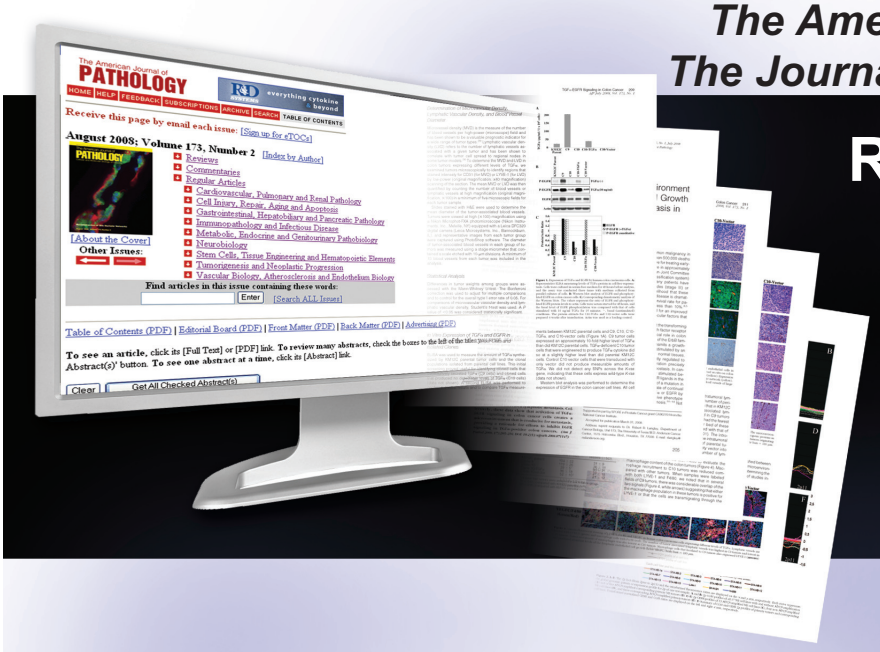
ANSWERS for CME Questions # 1-50

Volume/Issue	Month	Questions #	Answers
1	January	1-9	1b, 2d, 3e, 4d, 5e, 6d, 7b, 8c, 9a
2	March	10-18	10a, 11b, 12d, 13c, 14c, 15a, 16e, 17d, 18c
3	May	19-26	19e, 20a, 21d, 22a, 23b, 24c, 25d, 26e
4	July	27-34	27a, 28d, 29c, 30e, 31b, 32c, 33a, 34e
5	September	35-42	35e, 36a, 37c, 38d, 39c, 40e, 41b, 42b
6	November	43-50	43d, 44b, 45e, 46c, 47a, 48e, 49b, 50c

Explanations of answers to questions are divided by the issue in which the questions appeared.

The ASIP Journal CME Program



The American Journal of Pathology
The Journal of Molecular Diagnostics

Read the Articles... Take the Exams... Earn CME & SAM Credit!

The ASIP Journal CME programs for *The American Journal of Pathology (AJP)* and *The Journal of Molecular Diagnostics (JMD)* offer you a unique opportunity to earn up to 100 CME credits per year while renewing and updating your knowledge in the pathogenesis of disease and molecular diagnostics. The program consists of two components, the JMD CME Program in Molecular Diagnostics and the AJP CME Program in Pathogenesis. Participants can elect to participate in each program component individually or participate in both components simultaneously. The ASIP Journal CME Programs are also approved for Self-Assessment Module (SAM) credit with The American Board of Pathology.

JMD CME Program in Molecular Diagnostics

The **JMD CME Program in Molecular Diagnostics** provides ***The Journal of Molecular Diagnostics (JMD)*** readership with an opportunity to earn CME credit while renewing and updating their knowledge in the latest advances in molecular diagnostics. This program consists of a series of questions based on selected articles in the 2010 issues of *JMD*.

■ **Objectives -** Participants of the **JMD CME Program in Molecular Diagnostics** should be able to demonstrate an increase in, or confirmation of, their knowledge of the latest advances in molecular diagnosis and prognosis and understanding of molecular pathogenesis of disease that can lead to improvements in human health after reviewing specific articles in ***The Journal of Molecular Diagnostics (JMD)*.**

■ **Participants -** This program is specifically developed for trainees, clinicians and researchers interested in the molecular basis of disease and the application of nucleic acid and protein assays for diagnostic and prognostic analysis of disease.

■ **Examinations -** Each issue of *JMD* will include an online examination comprised of questions based on articles appearing in that particular issue. To receive credit for this journal-based CME activity, participants must answer questions based on selected articles in Volume 12 of *JMD* (calendar year 2010) and achieve a cumulative score of at least 75% (correct answers to at least 38 of the 50 questions in the annual program) in addition to completing an evaluation form.

AJP CME Program in Pathogenesis

The **AJP CME Program in Pathogenesis** provides ***The American Journal of Pathology (AJP)*** readership with a unique opportunity to earn CME credit while renewing and updating their knowledge in the mechanisms of disease. This program consists of a series of questions based on selected articles in the 2010 issues of *AJP*.

■ **Objectives -** Participants of the **AJP CME Program in Pathogenesis** should be able to demonstrate an increase in, or confirmation of, their knowledge of the pathogenesis of disease that can lead to improvements in human health after reviewing specific articles in ***The American Journal of Pathology (AJP)*.**

■ **Participants -** This program is specifically developed for trainees, clinicians and researchers investigating the mechanisms of disease who wish to advance their current knowledge of the cellular and molecular biology of disease.

■ **Examinations -** Each monthly issue of *AJP* will include an online examination comprised of questions based on articles appearing in that particular issue. To receive credit for this journal-based CME activity, participants must answer questions based on selected articles in Volume 176 and 177 of *AJP* (calendar year 2010) and achieve a cumulative score of at least 75% (correct answers to at least 38 of the 50 questions in the annual program) in addition to completing an evaluation form.

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Registration Rates*: AMP, ASIP, ISBER Member Rates - Single Program Component (AJP or JMD) $150/year, Non-Member Rates - $225/year**
AMP, ASIP, ISBER Member Rates - Full Program (AJP and JMD) $300/year, Non-Member Rates - $450/year**

*Note: The ASIP Journal CME Program includes both **The AJP CME Program in Pathogenesis** and the **JMD CME Program in Molecular Diagnostics**. Participants may select either one or both components, each of which is designated for a maximum of *50 AMA PRA Category 1 Credit(s)*™ .
AMP members are eligible for the Member Registration rate for the **JMD CME Program in Molecular Diagnostics only.

Register for the 2010 Journal CME Programs Online at
www.asip.org/CME/journalCME.htm

ASIP 2010 Journal CME Program

***AJP* CME Program in Pathogenesis, Volumes 176 and 177, 2010**
***JMD* CME Program in Molecular Diagnostics, Volume 12, 2010**
Director of CME Programs: Mark E. Sobel, MD, PhD

2010 Registration Form

Name		
Degree		
Institution		
Department		
Address		
City		State/Province
ZIP/Postal Code		Country
Telephone		Fax
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Registration Rates	**Please select program component(s) you wish to register for:** ❑ ***AJP*** ❑ Member Rates (ASIP & ISBER) - $150/year ❑ Non-Member Rates - $225/year ❑ ***JMD*** ❑ Member Rates (AMP*, ASIP & ISBER) - $150/year ❑ Non-Member Rates - $225/year *AMP members are eligible for the Member Registration rate for the *JMD* CME Program in Molecular Diagnostics only.	
Payment	❑ Check Enclosed Charge to Credit Card: ❑ VISA ❑ American Express ❑ MasterCard	
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Mail or Fax the Registration Form to the ASIP Office at:

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9650 Rockville Pike, Bethesda MD 20814-3993 (USA)
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2009 ASIP Journal CME Program

2009 JMD CME Program in Molecular Diagnostics

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

***The Journal of Molecular Diagnostics*, Volume 11, No. 1 (January 2009)**

http://jmd.amjpathol.org

http://www.asip.org/CME/journalCME.htm

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

ANSWERS for CME Questions # 1-9

1b, 2d, 3e, 4d, 5e, 6d, 7b, 8c, 9a

1. Mutations in the BCR-ABL tyrosine kinase are present in both chronic myelogenous leukemia (CML) and Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL). Based on the referenced Special Article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2009 11:4-11; DOI:10.2353/jmoldx.2009.080095; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. ATP-competitive kinase inhibitors that block BCR-ABL tyrosine kinase activity, such as imatinib mesylate (Gleevec), can induce a sustainable response in both CML and Ph+ ALL patients. **This statement is TRUE. However, resistant clones with mutations in the BCR-ABL kinase domain (KD) are a major mediator of disease recurrence.**
b. BCR-ABL kinase domain (KD) mutations are found in nearly 90% of patients with chronic phase CML who develop resistance to imatinib. **This statement is NOT TRUE. Only 30% to 50% of patients with chronic-phase CML who develop resistance to imatinib have BCR-ABL KD mutations. Other less common mechanisms of resistance include *BCR-ABL* gene amplification, BCR-ABL overexpression, alterations in drug efflux kinetics, up-regulation of other kinase pathways, and rare *BCR-ABL* mutations outside of the KD.**
c. *BCR-ABL* KD mutations occur in 80% to 90% of Ph+ ALL cases who have been previously treated with imatinib. **This statement is TRUE. *BCR-ABL* KD mutations are more prevalent in tyrosine kinase inhibitor (TKI)-treated Ph+ ALL patients than in CML patients.**
d. The identification of *BCR-ABL* KD mutations does not directly correlate with prognosis. **This statement is TRUE. Some studies have shown no difference in progression-free survival in TKI-resistant CML in the presence or absence of a *BCR-ABL* KD mutation. Use of more potent kinase inhibitors, however, can often overcome imatinib resistance due to KD mutations.**
e. CML and Ph+ ALL patients without clinical evidence of resistant disease occasionally have *BCR-ABL* KD mutations. **This statement is TRUE. *BCR-ABL* KD mutation screening is only recommended for chronic-phase CML patients with inadequate initial response to TKIs or patients with evidence of loss of response.**

2. At least 70 different mutations involving 57 different amino acids have been reported in the *BCR-ABL* kinase domain (KD). Based on the referenced Special Article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2009 11:4-11; DOI:10.2353/jmoldx.2009.080095; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Of all reported imatinib-resistant mutations, 15 amino acid substitutions account for 80% to 90% of mutations, and 7 mutated codons (G250, Y253, E255, T315, M351, F359, and H396) account for a cumulative 60% to 70% of mutations. **This statement is TRUE. The more common mutations cluster to one of four "hot spots" within the BCR-ABL KD, namely 1) the ATP-binding P-loop (amino acids 248-256), 2) the imatinib-binding region (amino acids 315-317), 3) the catalytic domain (amino acids 350-363), or 4) the activation (A)-loop (amino acids 381-402).**
b. Newer generation tyrosine kinase inhibitors (TKIs), such as dasatinib and nilotinib, preferentially select for certain mutations. **This statement is TRUE. Dasatinib use often selects for mutations at amino acids 299 (V299L), 315 (T315I), and 317 (F317L/I), and nilotinib preferentially selects for certain mutations in the P-loop (G250E, Y253H, E255K), as well as T315I or F311I.**
c. Different BCR-ABL KD mutations confer different degrees of resistance to treatment. **This statement is TRUE. Some mutations confer only low-level resistance that may respond to imatinib dose escalation (eg M351T) whereas others confer high-level resistance to both imatinib and other TKIs (eg T315I, Y253H, E255K).**
d. The spectrum of resistance mutations to the second-generation kinase inhibitors is less restricted than the spectrum of mutations seen following imatinib treatment. **This statement is NOT TRUE. Utilization of dasatinib and nilotinib leads to a more restricted mutation spectrum than that seen with imatinib.**
e. The development of BCR-ABL KD mutations may be part of the natural history of the disease. **This statement is TRUE. Mutations are more prevalent in patients with more advanced disease and are still often detectable both in pre-therapeutic samples and in patients with a complete cytogenetic response to TKI therapy.**

3. In addition to point mutations, other alterations in *BCR-ABL* have been reported in resistant patients. Based on the referenced Special Article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2009 11:4-11; DOI:10.2353/jmoldx.2009.080095; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Alternate splicing, insertions, deletions and/or duplications in *BCR-ABL* were observed by 58% of surveyed clinical laboratories. **This statement is TRUE. These alterations were observed in 7/12 laboratories surveyed.**
b. The most common non-point mutation reported was a 35-base pair intronic insertion at the exon 8/9 junction. **This statement is TRUE. Translation of this mutant would produce a BCR-ABL protein with an insertion of 10 amino acids followed by a stop codon.**
c. Exons 4, 7, or 8 have each been reported as lost due to alternate splicing of *BCR-ABL*. **This statement is TRUE. These alterations were reported by 5 laboratories.**
d. A Leu 248_Cys475 deletion was found by three laboratories at a frequency of approximately 2%. **This statement is TRUE. In addition, Arg316fs was reported by two laboratories, and Leu248_Lys274del, Met318_Thr319delinsLeu, and Ser385_Leu445del were also reported.**
e. Grossly altered transcripts with large deletions or early termination codons would be predicted to yield overactive BCR-ABL kinase activity. **This statement is NOT TRUE. Although the significance of grossly altered transcripts is unclear, many would be expected to lack active BCR-ABL kinase activity. Such truncated proteins arising from alternatively spliced transcripts may act as dominant-negative inhibitors of the full-length BCR-ABL.**

4. Hypertrophic cardiomyopathy (HCM) exhibits a relatively high incidence of sudden cardiac death. Based on the Commentary and related article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2009 11:12-16; DOI:10.2353/jmoldx.2009.080138 and J Mol Diagn 2009 11:35-41; DOI:10.2353/jmoldx.2009.080082; the authors of the referenced articles did not disclose any potential conflicts of interest.]

a. HCM is an autosomal dominant disease. **This statement is TRUE. HCM was the first myocardial infliction for which a genetic basis was identified.**
b. HCM is characterized by left ventricular hypertrophy, impaired myocardial contraction, impaired left ventricular diastolic function, and reduced exercise tolerance. **This statement is TRUE. Myocardial hypertrophy in HCM is often asymmetric with a diffuse or segmental pattern of left ventricle thickening.**
c. HCM is clinically variable, with some patients remaining asymptomatic and others experiencing the most serious complications. **This statement is TRUE. The heterogeneity of HCM symptoms complicates the management of patients with HCM.**
d. Nearly all patients with HCM have left ventricular outflow tract obstruction. **This statement is NOT TRUE. HCM can be either obstructive or nonobstructive. Twenty-five percent of HCM patients have a left ventricular outflow tract obstruction, adversely affecting prognosis.**
e. Current treatments focus on relieving the symptoms of HCM. **This statement is TRUE. Treatments include both lifestyle changes and pharmacological therapy.**

5. Angiotensin II plays an important role in the development of hypertrophic cardiomyopathy (HCM). Based on the Commentary and related article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2009 11:12-16; DOI:10.2353/jmoldx.2009.080138 and J Mol Diagn 2009 11:35-41; DOI:10.2353/jmoldx.2009.080082; the authors of the referenced articles did not disclose any potential conflicts of interest.]

a. Polymorphisms in the renin-angiotensin-aldosterone system may affect the severity of HCM. **This statement is TRUE. Gene polymorphisms of the angiotensin-converting enzyme (ACE) and angiotensin II type 1 receptor (ATR-1) are associated with the severity of hypertrophy and the prognosis of HCM patients.**
b. In a mouse model of human HCM, angiotensin II blockage through the use of the angiotensin II type 1 receptor (ATR-1) antagonist losartin decreased the extent of interstitial fibrosis and collagen synthesis compared with placebo. **This statement is TRUE. Treatment with the ATR-1 antagonists may attenuate myocardial fibrosis and hypertrophy.**
c. The ATR-1 inhibitor valsartan reduced collagen synthesis in HCM patients. **This statement is TRUE. After 12 months of valsartan treatment, no favorable effects were observed on left ventricle diastolic function, filling pressures, or degrees of hypertrophy.**
d. Angiotensin-converting enzyme (ACE) activity varies greatly among individuals. **This statement is TRUE. ACE activity variability is mainly due to an insertion/deletion polymorphism in intron 16.**
e. Inhibition of ACE has no effect on myocardial hypertrophy. **This statement is NOT TRUE. Inhibition of either ACE or ATR-1 decreased myocardial hypertrophy in hypertensive patients or in patients post-myocardial infarction.**

6. Hypertrophic cardiomyopathy (HCM) is caused by mutations in the sarcomeric proteins. Based on the Commentary and related article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2009 11:12-16; DOI:10.2353/jmoldx.2009.080138 and J Mol Diagn 2009 11:35-41; DOI:10.2353/jmoldx.2009.080082; the authors of the referenced articles did not disclose any potential conflicts of interest.]

a. Greater than 400 different mutations have been identified in genes for proteins in the sarcomere and myofilaments. **This statement is TRUE. Most of the known alterations are missense mutations, with a single amino acid residue substituted for another.**
b. The majority of HCM molecular defects lie in genes encoding functional and regulatory sarcomeric proteins such as β-myosin heavy chain (β -MHC), actin, cardiac troponin T and I (cTnT and cTnI), and tropomyosin. **This statement is TRUE. Mutations can also be found in structural proteins, ie myosin-binding protein C (MYBPC) and titin.**
c. Different phenotypic and functional outcomes in patients with HCM may be caused by specific gene mutations. **This statement is TRUE. Mutations in β-MHC appear to result in disease at a younger age, with more pronounced hypertrophy and a higher risk of sudden cardiac death when compared with HCM caused by mutations in MYBPC or α-tropomyosin genes.**
d. Hearts from subjects harboring a TnT mutation exhibit a severe hypertrophic phenotype and a low incidence of sudden cardiac death. **This statement is NOT TRUE. Patients with a TnT mutation have mild hypertrophy but a high risk for sudden cardiac death.**
e. Although HCM is a monogenic disease, the final phenotype is likely influenced by a complex blend of the primary mutation, other gene alterations, and the surrounding environment. **This statement is TRUE. Primary mutation, other gene alterations, and the surrounding environment should all be taken into consideration when treating HCM.**

7. The long-term use of the angiotensin II type 1 receptor (ATR-1) blocker candesartan is associated with regression of left ventricular hypertrophy. Based on the Commentary and related article, select the ONE statement that is NOT TRUE. [See J Mol Diagn 2009 11:12-16; DOI:10.2353/jmoldx.2009.080138 and J Mol Diagn 2009 11:35-41; DOI:10.2353/jmoldx.2009.080082; the authors of the referenced articles did not disclose any potential conflicts of interest.]

a. Patients treated with candesarten had a significant reduction in left ventricle thickness and mass as well as improved systolic and diastolic function when compared with those receiving placebo. **This statement is TRUE. Candesartan induced regression of left ventricular hypertrophy and improved left ventricular function and exercise tolerance in patients with nonobstructive HCM.**
b. The left ventricle ejection fraction was significantly improved in candesartan-treated patients. **This statement is NOT TRUE. There was no significant difference in the left ventricle ejection fraction between the placebo and candesartan-treated groups.**
c. Specific genetic mutations can be used to predict the efficacy of ATR-1 inhibition in HCM. **This statement is TRUE. Heterogeneous response to candesartan treatment is partly dependent on the specific sarcomeric protein gene mutation.**
d. The findings of the referenced study, which compared the effectiveness of candesartan in patients with different mutations, are limited by the fact that neither ACE nor AT1-R polymorphisms were assessed. **This statement is TRUE. Since the carriers of the ACE D or AT1-R C alleles may experience the greatest benefit from angiotensin II blockade, the information on these polymorphisms could help in sleecting patients for AT1-R antagonist therapy.**
e. Higher levels of hypertrophy regression were observed in response to candesartan in patients with β-MHC and MYBPC mutations. **This statement is TRUE. Patients with β-MHC mutations showed the greatest decrease in left ventricular mass, followed by MYBPC patients, in response to cardesartan. No regression of hypertrophy was observed in patients with the cTnI gene mutation.**

8. *Neisseria gonorrhoeae* and *N. meningitidis* are difficult to definitively identify due to the small number of taxonomic differences between species. Based on the referenced article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2009 11:75-86; DOI:10.2353/jmoldx.2009.080079; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Gonococcal infection, caused by *N. gonorrhoeae,* is one of the most common sexually transmitted diseases in the world. **This statement is TRUE. *N. gonorrhoeae* is estimated to infect more than 60 million people each year.**
b. *N. meningitis,* common flora of the nose and throat, can lead to bacterial meningitis. **This statement is TRUE. Although the highest burden of disease exists in Africa, epidemics have occurred in Asia, Europe, and the Americas in the last 30 years.**
c. Real-time PCR is the primary conventional diagnostic method for *Neisseria* species. **This statement is NOT TRUE. Enzymatic tests are used as the primary means to differentiate *Neisseria* species, but no single method is currently recommended for a definitive identification.**
d. Prolyliminopeptidase is not universally present in *N. gonorrhoeae.* **This statement is TRUE. Prolyliminopeptidase-negative *N. gonorrhoeae* strains also exist, making prolyliminopeptidase testing inconclusive.**
e. Microbiologic identification methods based on morphological and biochemical characteristics are time consuming, error prone, and laborious. **This statement is TRUE. Although *N. gonorrhoeae* and *N. meningitidis* are relatively straightforward to identify, speciation of many of the nonpathogenic strains is not always possible.**

9. Direct bacterial profiling (DBP) by means of matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry provides a new approach for rapid and accurate identification of microbes. Based on the referenced article, select the ONE statement that is NOT TRUE: [See J Mol Diagn 2009 11:75-86; DOI:10.2353/jmoldx.2009.080079; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. MALDI-TOF requires a large quantity of starting material to classify microorganisms. **This statement is NOT TRUE. MALDI-TOF requires only a small amount of biological material. Only one bacterial colony is sufficient to run MALDI-TOF analysis.**
b. MALDI-TOF is a fast and easy protocol. **This statement is TRUE.**
c. MALDI-TOF has high species specificity. **This statement is TRUE. In spite of the close relationship between *N. gonorrhoeae* and *N. meningitides,* only six peaks were found to be identical between these species.**
d. MALDI-TOF can be used to identify microorganisms directly from whole cells without preliminary separation and extraction. **This statement is TRUE. Other protocols that have been suggested include different methods of protein extraction and utilization of nonionic surfactants to expand the upper mass range for bacterial proteins obtained from whole-cell MALDI-TOF mass spectrometry analysis.**
e. *Neisseria* species can be clearly distinguished by MALDI-TOF. **This statement is TRUE. *Neisseria* species have low intra-species variability, high inter-species heterogeneity, and high mass spectrometric reproducibility, making MALDI-TOF a suitable approach for species differentiation.**

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2009 JMD CME Program in Molecular Diagnostics

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

***The Journal of Molecular Diagnostics*, Volume 11, No. 2 (March 2009)**

http://jmd.amjpathol.org

http://www.asip.org/CME/journalCME.htm

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

ANSWERS for CME Questions # 10-18
10a, 11b, 12d, 13c, 14c, 15a, 16e, 17d, 18c

10. DNA methylation levels serve as a biomarker for early cancer diagnosis, risk assessment, and therapy responsiveness. Based on the referenced Technical Advances article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:102-108; DOI:10.2353/jmoldx.2009.080109; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Promoter hypomethylation in cancerous cells is associated with transcriptional silencing and loss of gene expression. **This statement is NOT TRUE. Promoter hypermethylation is associated with transcriptional silencing and loss of gene expression.**
b. Sodium bisulfite treatment of genomic DNA facilitates the determination of methylation status. **This statement is TRUE. Sodium bisulfite converts cytosine, but not 5-methylcytosine, to uracil. PCR amplication is then used to establish cytosine methylation status.**
c. Methylation-specific PCR, or MSP, is the most widely used PCR-based technique for detection of methylation. **This statement is TRUE. This assay uses primers restricted to methylated, bisulfite-modified DNA.**
d. MSP only provides qualitative data on methylation status. **This statement is TRUE. MSP cannot distinguish between low and high levels of methylation, and low levels of methylation may not be biologically important.**
e. Fluorescence-based real-time PCR analysis (such as MethyLight) represents a quantitative and high-throughput method of determining methylation levels. **This statement is TRUE. This method, however, is neither cost-effective nor fast enough for routine clinical diagnosis.**

11. High-resolution melting curve analysis (HRM) is a relatively new approach for sensitive and high-throughput assessment of methylation. Based on the referenced Technical Advances article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:102-108; DOI:10.2353/jmoldx.2009.080109; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. HRM relies on the melting properties of DNA in solution. **This statement is TRUE. Highly methylated bisulfite-treated DNA has a different melting temperature than bisulfite-treated DNA with no or low methylation.**
b. HRM requires longer probes than those used in MethyLight assays. **This statement is NOT TRUE. HRM does not require the use of probes. The use of probes in MethyLight assays increases the cost of experiments compared with HRM.**
c. MethyLight, but not HRM, requires normalization against a reference gene. **This statement is TRUE. HRM experiments are simpler to design and implement than MethyLight experiments.**
d. HRM analysis can potentially detect all of the methylated sites within the target sequence whereas MethyLight is restricted to sites bound by the primers and probes. **This statement is TRUE. HRM analysis can provide a more accurate depiction of the methylation status of a DNA template.**
e. HRM can distinguish heterogeneous from homogeneous methylation by the shape of the melting curve. **This statement is TRUE. MethyLight cannot distinguish between homogeneous and heterogeneous methylation.**

12. HRM-based methylation analysis is applicable to the examination of formalin-fixed paraffin-embedded (FFPE) tissues. Based on the referenced Technical Advances article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:102-108; DOI:10.2353/jmoldx.2009.080109; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. HRM was used to assess *MGMT* and *APC* promoter methylation on DNA from fresh and FFPE human cancer cell lines as well as FFPE colorectal tumor specimens. **This statement is TRUE. FFPE tissues are the largest source of clinical material and are often difficult to analyze by PCR due to the low quality of the DNA template.**
b. HRM methylation results were consistent among dilutions and replicates. **This statement is TRUE. Consistency was evaluated using a methylated DNA dilution matrix as well as clinical samples.**
c. HRM results were consistent between fresh and FFPE samples. **This statement is TRUE. HRM can therefore be used to accurately examine FFPE samples.**
d. HRM methylation ratios increased with greater quantities of DNA template. **This statement is NOT TRUE. No differences were observed in methylation ratios using different quantities of DNA.**
e. There was a high correlation between HRM and MethyLight results. **This statement is TRUE. All HRM results were validated with MethyLight assays.**

13. Fluorescence *in situ* hybridization (FISH) may be used to detect bladder cancer by examining abnormal cells in urine. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:148-154; DOI:10.2353/jmoldx.2009.080096; one of the authors is listed as a co-inventor on a patent; that author and the Mayo Clinic receive royalties from the sale of the FISH probe set used in this study. In addition, one author receives grant support from Abbott Molecular Inc. to develop FISH assays for the detection of malignant cells in cytologic specimens. No other co-authors of this study have potential conflicts of interest regarding any of the products used in this study.]

a. Quantitative FISH is as specific, and more sensitive, than routine cytology for detecting bladder cancer in urine. **This statement is TRUE. FISH can frequently detect recurrent bladder cancer before it is clinically evident by cytology.**
b. A positive urine FISH result for the detection of bladder cancer is defined as ≥ 4 cells with a polysomic signal pattern (ie, cells with gains of two or more of the four chromosomal targets). **This statement is TRUE. A positive FISH result is based on the actual number of abnormal cells counted and not the percentage of abnormal cells.**
c. The UroVysion probe set includes a fluorescently labeled DNA probe specific to the 6p21 band, near the location of the *P53* tumor suppressor gene. **This statement is NOT TRUE. The UroVysion probe set consists of fluorescently labeled DNA probes specific to the pericentromeric regions of chromosome 3, 7, and 17 and the 9p21 band, near the location of the *P16* tumor suppressor gene.**
d. At one year post FISH analysis, approximately 25% of patients in the 0% FISH abnormal category had a cancer diagnosis. **This statement is TRUE. Greater than 90% of patients with >30% abnormal cells developed recurrent tumor.**
e. Caucasians accounted for over 99% of the patient population in the current study, and 85% of the population was male. **This statement is TRUE. Further studies are needed to determine if the outcomes of the study would differ significantly with a non-Caucasian or female population.**

14. The percentage of abnormal cells detected by FISH predicts cancer recurrence and progression in patients with a history of non-invasive bladder cancer. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:148-154; DOI:10.2353/jmoldx.2009.080096; one of the authors is listed as a co-inventor on a patent; that author and the Mayo Clinic receive royalties from the sale of the FISH probe set used in this study. In addition, one author receives grant support from Abbott Molecular Inc. to develop FISH assays for the detection of malignant cells in cytologic specimens. No other co-authors of this study have potential conflicts of interest regarding any of the products used in this study.]

a. Approximately 70% of bladder cancers diagnosed in 2007 were non-muscle invasive tumors. **This statement is TRUE. There were an estimated 67,160 newly diagnosed bladder cancers in 2007.**
b. After initial diagnosis, up to 70% of patients with non-muscle invasive bladder cancer have recurrent tumors. **This statement is TRUE. Approximately 10-30% of these tumors will then progress to a more dangerous muscle invasive cancer.**
c. Kaplan-Meier analysis revealed an increased time to tumor recurrence in female patients. **This statement is NOT TRUE. Kaplan-Meier analysis revealed a decreased time to tumor recurrence in patients who were female, had a positive cytoscopy result, had no therapy after FISH, or had a history of a CIS or T1 stage tumor prior to FISH.**
d. The percentage of abnormal cells by FISH, patient age, and male sex are significantly associated with time to cancer recurrence. **This statement is TRUE. Treatment and history of TIS/T1-stage tumor is also associated with time to cancer recurrence.**
e. The percentage of abnormal cells by FISH was significantly associated with the time to development of muscle invasive bladder cancer. **This statement is TRUE. There was a 1.8% increased risk of having muscle invasive cancer for every 1% increase in the percentage of abnormal cells by FISH.**

15. False-positive FISH results can lead to costly, invasive, and potentially dangerous evaluation and treatment in patients that do not have disease. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:148-154; DOI:10.2353/jmoldx.2009.080096; one of the authors is listed as a co-inventor on a patent; that author and the Mayo Clinic receive royalties from the sale of the FISH probe set used in this study. In addition, one author receives grant support from Abbott Molecular Inc. to develop FISH assays for the detection of malignant cells in cytologic specimens. No other co-authors of this study have potential conflicts of interest regarding any of the products used in this study.]

a. The data suggest that patients with as low as 0.5% abnormal cells by FISH analysis should be evaluated for recurrent tumor by random biopsies, even in the absence of clinical or cytoscopic evidence of tumor. **This statement is NOT TRUE. The data suggest that patients with ≥5% abnormal cells should possibly be evaluated for recurrent tumor even in the absence of clinical or cytoscopic evidence of tumor. False-positive FISH results in this study primarily occurred in positive patients (≥ 4 cells with a polysomic signal pattern) with only 1-4% abnormal cells. Only 50% of positive patients with 1-4% abnormal cells developed recurrent tumor.**
b. Misdiagnosis by FISH can occur because cells may appear polysomic due to signal splitting. **This statement is TRUE.**
c. Misdiagnosis by FISH can occur in the presence of a few tetrasomic cells with four copies for each of the four probes. **This statement is TRUE. This can occur with normal cells in the G2 or M phase of the cell cycle.**
d. Unrecognized overlapping cells may result in a false-positive FISH result. **This statement is TRUE. Low-level positive (1-4% abnormal) FISH specimens should be reviewed by a second technologist to decrease the chance for false-positive diagnosis.**
e. Some apparent false-positive FISH results may be explained by eradication of tumor as a result of treatment following the initial positive FISH result. **This statement is TRUE. Cox proportional hazard analyses show that treatment following FISH was an independent predictor of a decreased chance of bladder cancer recurrence and progression of muscle-invasive disease.**

16. Genetic tests have become a part of standard care; however, the types of tests ordered vary by practice. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:162-171; DOI:10.2353/jmoldx.2009.080130; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. The 2001 publication of a guideline for preconception and prenatal carrier screening for cystic fibrosis prompted a significant increase in referrals by obstetrician-gynecologists (OB-GYNs) for cystic fibrosis carrier testing. **The statement is TRUE. The three clinician groups examined, OB-GYNs, pediatricians, and family practitioners, all frequently ordered cystic fibrosis carrier testing.**
b. Family practitioners do not often order genetic testing for fragile X syndrome. **This statement is TRUE. OB-GYNs and pediatricians were much more likely to order genetic testing for fragile X syndrome.**
c. Hereditary hemochromatosis type 1 is commonly screened for by family practitioners and pediatricians. **This statement is TRUE. OB-GYNs are less likely to screen for *HFE* mutations.**
d. *BRCA1 and BRCA2* mutation screening was performed by both OB-GYNs and family practitioners. **This statement is TRUE. Pediatricians rarely ordered tests for familial *BRCA1* and *BRCA2* mutations.**
e. Although a different variety of genetic tests were ordered depending on practice, OB-GYNs, pediatricians, and family practitioners all requested similar numbers of tests. **This statement is NOT TRUE. Pediatricians order, on average, one genetic test per month; OB-GYNs order genetic tests daily; family practitioners order genetics tests a few times a year.**

17. Information derived from genetic tests should be effectively communicated from laboratory to clinical professionals and applied appropriately to patient care. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:162-171; DOI:10.2353/jmoldx.2009.080130; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Only 22.2% of clinicians could correctly answer a question about residual risk and race/ethnicity in cystic fibrosis genetic testing. **This statement is TRUE. Race/ethnicity is essential in understanding residual risk for individual patients in cystic fibrosis testing.**
b. A 1997 report estimated that physicians ordering a genetic test for familiar adenomatous polyposis erroneously interpreted a negative result as ruling out disease. **This statement is TRUE. The test was not able to detect the full spectrum of disease-associated mutations.**
c. Approximately 25% of *BRCA1/2* test requests lacked information about the patient and/or their family. **This statement is TRUE. Familial information is important in interpreting genetic risk information related to *BRCA* and *BRCA2.***
d. Laboratories are consistent in how they report residual risk and uncertainties in test interpretation. **This statement is NOT TRUE. Laboratories vary in how they report residual risk and uncertainties in diagnostic test interpretations.**
e. Staff members lacking formal medical training often fill out test requisition forms, review results, and communicate with patients about test results. **This statement is TRUE. Factors in both laboratory and clinical settings such as the collection and use of patient- and family-specific information, variation and formation of test requisitions and results reports, and the competency of medical staff lacking specialized knowledge of genetics may compromise test results.**

18. A standardized clinician-friendly laboratory report was proposed for molecular genetic tests. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:162-171; DOI:10.2353/jmoldx.2009.080130; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Input was solicited from pediatricians, OB-GYNs, and family practitioners to generate the standardized clinician-friendly laboratory report. **This statement is TRUE. Laboratory technicians and policy, education, information technology, and third-party payer professionals were also consulted. Reports were modeled based on workgroup discussions, previous studies, and professional guidelines.**
b. The proposed laboratory report covers patient-specific information, test-specific information, test result interpretation, and guidance for future steps. **This statement is TRUE.**
c. Participants preferred a longer, more detailed, report to a short report. **This statement is NOT TRUE. Participants preferred a shorter to a longer report.**
d. Patient-specific interpretation in the laboratory reports was suggested to aid physicians and medical staff in properly communicating the implications of genetic tests. **This statement is TRUE. Reports should clearly state the significance of the results and provide guidance for next steps.**
e. Clinicians preferred graphic representation to descriptive wording when possible. **This statement is TRUE. An example would be including a table of residual risk assessments for various racial/ethnic groups.**

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ANSWERS for CME Questions # 19-26
19e, 20a, 21d, 22a, 23b, 24c, 254b, 26e

19. Cystic fibrosis is an autosomal recessive hereditary disease. Based on the referenced Commentary and related articles and Consultation in Molecular Diagnostics, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:173-175; DOI:10.2353/jmoldx.2009.090024; J Mol Diagn 2009 11:186-193; DOI:10.2353/jmoldx.2009.080149; three of the authors are employees of Quest Diagnostics, one author is an employee of Fullerton Genetics, and one author is an employee of Genzyme Corporation; J Mol Diagn 2009 11:211-215; DOI:10.2353/jmoldx.2009.080106; the authors are employees of Quest Diagnostics or Focus Diagnostics; and J Mol Diagn 2009 11:253-256; DOI:10.2353/jmoldx.2009.080117; the authors are employees of Quest Diagnostics and three of them hold stock in the company.]

a. Cystic fibrosis leads to pulmonary and gastrointestinal complications as well as male infertility. **This statement is TRUE.**
b. Cystic fibrosis is one of the most common autosomal recessive disorders among Caucasians. **This statement is TRUE. The estimated cystic fibrosis incidence among Caucasians is 1 in 3,300 live births. Approximately 1,000 new cases are reported each year.**
c. Mutations in the *CFTR* gene cause the development of cystic fibrosis. **This statement is TRUE. The *CFTR* gene encodes the cystic fibrosis transmembrane conductance regulator (CFTR).**
d. Mutations in *CFTR* result in a chloride ion transport channel defect. **This statement is TRUE. CFTR transports chloride ions across the epithelial cell membrane.**
e. A single insertional mutation in *CFTR* is responsible for 95% of clinical cystic fibrosis cases. **This statement is NOT TRUE. There is tremendous mutational heterogeneity in the *CFTR* gene. Over 1,500 mutations have been identified in *CFTR*.**

20. Genetic testing is used for cystic fibrosis diagnosis and carrier screening. Based on the referenced Commentary and related articles and Consultation in Molecular Diagnostics, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:173-175; DOI:10.2353/jmoldx.2009.090024; J Mol Diagn 2009 11:186-193; DOI:10.2353/jmoldx.2009.080149; three of the authors are employees of Quest Diagnostics, one author is an employee of Fullerton Genetics, and one author is an employee of Genzyme Corporation; J Mol Diagn 2009 11:211-215; DOI:10.2353/jmoldx.2009.080106; the authors are employees of Quest Diagnostics or Focus Diagnostics; and J Mol Diagn 2009 11:253-256; DOI:10.2353/jmoldx.2009.080117; the authors are employees of Quest Diagnostics and three of them hold stock in the company.]

a. The core screening panel recommended by the American College of Medical Genetics (ACMG) and the American College of Obstetricians and Gynecologists (ACOG) for cystic fibrosis is 45 mutations. **This statement is NOT TRUE. The core screening panel for cystic fibrosis is 23 mutations. Many laboratories use even larger "expanded" panels for diagnosis and screening.**
b. Positive mutation controls have been established for the alleles in the ACMG core panel. **This statement is TRUE. Mutations controls were artificially constructed using recombinant DNA methods and site-directed mutagenesis.**
c. Differences in allele frequencies in different ethnic and racial subsets of the population complicate cystic fibrosis genetic counseling. **This statement is TRUE. Another challenge to cystic fibrosis screening was the slow uptake from the screening providers, primary obstetricians.**
d. Cystic fibrosis genetic screening has significantly diminished the number of unwanted cystic fibrosis births. **This statement is TRUE.**
e. Professional guidelines for universal carrier screening of the reproductive age population have led to increased levels of cystic fibrosis screening. **This statement is TRUE. Cystic fibrosis carrier screening has become incorporated into routine prenatal care.**

21. Mutation controls need to be developed for the "expanded" panel of cystic fibrosis mutations in commercial assay platforms. Based on the referenced Commentary and related articles and Consultation in Molecular Diagnostics, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:173-175; DOI:10.2353/jmoldx.2009.090024; J Mol Diagn 2009 11:186-193; DOI:10.2353/jmoldx.2009.080149; three of the authors are employees of Quest Diagnostics, one author is an employee of Fullerton Genetics, and one author is an employee of Genzyme Corporation; J Mol Diagn 2009 11:211-215; DOI:10.2353/jmoldx.2009.080106; the authors are employees of Quest Diagnostics or Focus Diagnostics; and J Mol Diagn 2009 11:253-256; DOI:10.2353/jmoldx.2009.080117; the authors are employees of Quest Diagnostics and three of them hold stock in the company.]

a. Testing more than the ACMG core panel of mutations may be beneficial for diagnostic testing or newborn screening. **This statement is TRUE. Most laboratories consider the carrier screening panel sufficient for diagnostic or newborn testing; however, populations with particular ethnic mixes may benefit from expanded testing.**
b. Two out of four Food and Drug Administration-cleared cystic fibrosis assays test for more mutations than the recommended screening panel. **This statement is TRUE. Inclusion of additional cystic fibrosis alleles does not significantly increase the detection frequency in European Caucasians.**
c. Reference materials for the expanded panel of mutations could be used for test development and validation, lot-testing of new reagent batches, and for performance evaluation programs. **This statement is TRUE.**
d. In the absence of characterized DNA reference materials, laboratories must rely on healthy patient DNA samples. **This statement is NOT TRUE. In the absence of characterized DNA reference materials, laboratories must rely on residual patient specimens. Residual patient specimens are often difficult to find and not consistently available.**
e. Pratt et al (DOI:10.2353/jmoldx.2009.080149) developed 15 positive mutation controls and characterized publicly available cell lines for expanded cystic fibrosis mutation testing. **This statement is TRUE. These materials supplement the available characterized genomic DNA reference materials, which cover the recommended mutation screening panel.**

22. Multiplex testing platforms are subject to rare false results. Based on the referenced Commentary and related articles and Consultation in Molecular Diagnostics, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:173-175; DOI:10.2353/jmoldx.2009.090024; J Mol Diagn 2009 11:186-193; DOI:10.2353/jmoldx.2009.080149; three of the authors are employees of Quest Diagnostics, one author is an employee of Fullerton Genetics, and one author is an employee of Genzyme Corporation; J Mol Diagn 2009 11:211-215; DOI:10.2353/jmoldx.2009.080106; the authors are employees of Quest Diagnostics or Focus Diagnostics; and J Mol Diagn 2009 11:253-256; DOI:10.2353/jmoldx.2009.080117; the authors are employees of Quest Diagnostics and three of them hold stock in the company.]

a. Mutations that interfere with primer binding can only lead to false positive results. **This statement is NOT TRUE. Mutations that interfere with primer binding can also lead to false negative results if the other allele is normal.**
b. Hybridization probe binding can be blocked by mutations in probe binding sites. **This statement is TRUE. Failure of the hybridization probe to bind can result in allele dropout.**
c. Mutations may occur in restriction enzyme digestion sites. **This statement is TRUE. Restriction enzyme digestion sites are used to identify particular mutations.**
d. The American College of Medical Genetics (ACMG) screening panel controls for presumably benign polymorphisms that interfere with DNA probe hybridization for certain genuine mutations. **This statement is TRUE.**
e. The polymorphism F508C can interfere with probe hybridization to the normal F508 allele. **This statement is TRUE. This causes allele "dropout," causing a possible F508C and ΔF508 heterozygote to appear as a ΔF508 homozygote and generating a false positive result.**

23. Allele dropout can produce a false appearance of mutational homozygosity. Based on the referenced Commentary and related articles and Consultation in Molecular Diagnostics, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:173-175; DOI:10.2353/jmoldx.2009.090024; J Mol Diagn 2009 11:186-193; DOI:10.2353/jmoldx.2009.080149; three of the authors are employees of Quest Diagnostics, one author is an employee of Fullerton Genetics, and one author is an employee of Genzyme Corporation; J Mol Diagn 2009 11:211-215; DOI:10.2353/jmoldx.2009.080106; the authors are employees of Quest Diagnostics or Focus Diagnostics; and J Mol Diagn 2009 11:253-256; DOI:10.2353/jmoldx.2009.080117; the authors are employees of Quest Diagnostics and three of them hold stock in the company.]

a. Schwartz et al (DOI:10.2353/jmoldx.2009.080106) observed over 37 cases with unusual electropherogram profiles in a PCR/oligonucleotide ligation assay. **This statement is TRUE. The authors examined over 1,000,000 patient specimens in a large commercial reference library.**
b. Allele dropout was observed for only one mutation in the screening panel. **This statement is NOT TRUE. Allele dropout was observed for various mutations in the screening panel.**
c. Allele dropout was primarily caused by polymorphic variants at or under the oligonucleotide ligation site. **This statement is TRUE. This allelic dropout was therefore specific to the method used.**
d. Schwartz et al (DOI:10.2353/jmoldx.2009.080106) failed to observe allele dropout due to an interfering polymorphism under a PCR primer hybridization site. **This statement is TRUE. Such variants likely exist, however.**
e. It should be possible to rule out anomalous results by retesting with a different commercial screening platform. **This statement is TRUE. Retesting may require collaboration with another laboratory and cooperation from the recommending physician.**

24. One cause of allele dropout producing a false appearance of homozygosity is the presence of a large deletion in the opposite allele. Based on the referenced Commentary and related articles and Consultation in Molecular Diagnostics, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:173-175; DOI:10.2353/jmoldx.2009.090024; J Mol Diagn 2009 11:186-193; DOI:10.2353/jmoldx.2009.080149; three of the authors are employees of Quest Diagnostics, one author is an employee of Fullerton Genetics, and one author is an employee of Genzyme Corporation; J Mol Diagn 2009 11:211-215; DOI:10.2353/jmoldx.2009.080106; the authors are employees of Quest Diagnostics or Focus Diagnostics; and J Mol Diagn 2009 11:253-256; DOI:10.2353/jmoldx.2009.080117; the authors are employees of Quest Diagnostics and three of them hold stock in the company.]

a. The presence of a deletion involving one or more of the primer hybridization sites results in amplification failure for that particular product. **The statement is TRUE. The nonamplified, deleted, allele will therefore not be seen. Only the opposite allele will be analyzed and will appear homozygous.**
b. A large deletion can result either in a false negative or false positive homozygosity. **This statement is TRUE. The large deletion will result in the appearance of homozygosity of the opposite allele, whether normal or mutated.**
c. Allele dropout due to a large deletion was first noticed in DNA testing for autoimmune disorders. **This statement is NOT TRUE. Allele dropout due to a large deletion was first noticed in DNA testing for familial cancer mutations. 10-15% of BRCA mutations are deletions, which produce normal DNA sequencing results.**
d. The *CFTR*dele2,3(21 kb) accounts for approximately 4% of cystic fibrosis mutated chromosomes in people of Slavic origin. **This statement is TRUE. It accounts for 0.2% of cystic fibrosis mutated chromosomes in the United States population.**
e. In the case presented by Hantash et al (DOI:10.2353/jmoldx.2009.080117), a small homozygous deletion was observed by sequencing. **This statement is TRUE. Further experiments revealed a small exon 20 deletion and a larger 40 kb deletion on the opposite allele.**

25. The safe and effective dosage of warfarin is altered by the presence of single nucleotide polymorphisms (SNPs) in two genes. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:216-225; DOI:10.2353/jmoldx.2009.080123; one author is a consultant for ParagonDx, and one author is a consultant for Third Wave Technologies.]

a. Responses to warfarin are affected by genetic variability in the *CYP2C9* (cytochrome P450 2C9) and the *VKORC1* (vitamin K epoxide reductase complex) genes. **This statement is TRUE. Warfarin binds to and inhibits vitamin K epoxide reductase (VKOR), thereby inhibiting activation of vitamin K-dependent clotting factors.**
b. VKORC1 group B haplotypes H3 and H4 have increased sensitivity to warfarin. **This statement is NOT TRUE. VKORC1 group A haplotypes H1 and H2 have increased sensitivity to warfarin. A *VKORC1* SNP (1639/3673 A>G) defines *VKORC1* haplotypes H1 and H2.**
c. CYP2C9 is responsible for metabolism of >90% of S-warfarin, the more active enantiomer of warfarin. **This statement is TRUE.**
d. Two common allelic variants of *CYP2C9* with reduced enzymatic activity have been associated with reduced metabolism of warfarin, lower required doses of warfarin to achieve adequate anticoagulation, and increased risk of adverse events when beginning warfarin therapy. **This statement is TRUE. These two variants are CYP2C9*2 and CYP2C9*3.**
e. The Washington University and the UCSF algorithms demonstrated the best correlation between the predicted doses and the actual doses optimized in a population on long-term warfarin therapy. **This statement is TRUE. Several different dosing algorithms have been devised that incorporate the*VKORC1* and *CYP2C9* genotype, in addition to demographic and clinical information, to predict optimal warfarin dose. The four studied here were from Washington University, UCSF, Louisville, and Newcastle.**

26. Four platforms were compared for determining the relevant SNPs in both *Cyp2C9* and *VKORC1* that are associated with warfarin sensitivity. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:216-225; DOI:10.2353/jmoldx.2009.080123; one author is a consultant for ParagonDx, and one author is a consultant for Third Wave Technologies.]

a. All the methods evaluated screened for *CYP2C9* *2 and *3 and at least one of the relevant *VKORC1* SNPs. This statement is TRUE. **The platforms examined were Third Wave Invader® Plus, ParagonDx/Cepheid Smart Cycler, Idaho Technology LightCycler, and AutoGenomics INFINITI.**
b. The ParagonDx and the Idaho Technologies assays had the shortest hands-on-time and turnaround time. **This statement is TRUE. Data are collected from these assays directly from the instrument during or immediately after PCR.**
c. The Autogenomics assay requires specialized training to use, but less than one hour of hands-on-time. **This statement is TRUE. The INFINITY instrument from Autogenomics screened the greatest number of SNPs, but required the largest capital investment.**
d. The Third Wave assay was readily scalable to larger volumes. **This statement is TRUE. However, the Third Wave assay had the longest turnaround time.**
e. All genotyping methods demonstrated approximately 70% accuracy for identifying the pertinent SNPs. **This statement is NOT TRUE. All genotyping methods demonstrated greater than 95% accuracy for identifying the pertinent SNPs.**

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ANSWERS for CME Questions # 27-34
27a, 28d, 29c, 30e, 31b, 32c, 33a, 34e

27. CpG island methylation in gene promoter regions regulates gene expression. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:266-278; DOI:10.2353/jmoldx.2009.080125; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. CpG islands must be composed of at least 80% G+C content. **This statement is NOT TRUE. According to the most recent literature, CpG islands are defined as being at least 500 bp long with at least 55% G+C content.**
b. CpG islands in actively transcribed genes are unmethylated. **This statement is TRUE. Increased cytosine methylation is associated with reduced gene expression.**
c. Gene regulation by CpG methylation is involved in both development and aging. **This statement is TRUE. CpG methylation-mediated regulation is also involved in inflammatory and infection diseases and cancer.**
d. The authors recommend the development of standardized quality control materials for CpG assays and the adoption of standard reporting formats to facilitate the incorporation of CpG methylation assays as a routine component of clinical molecular diagnostics. **This statement is TRUE. Researchers are urged to identify and define the CpG sites analyzed in published reports by listing DNA sequences and/or a description of the location of the tested CpG site(s) in relation to the transcription start site.**
e. CpG methylation tests are clinically useful. **This statement is TRUE. The levels of CpG methylation can be used to sub-classify tumors, predict responses to chemotherapy, and assess the effects of methylating and demethylating therapies.**

28. Analysis of CpG methylation includes amplification and differentiation of methylated and unmethylated sequences. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:266-278; DOI:10.2353/jmoldx.2009.080125; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Polymerase chain reaction (PCR) is first used to amplify the targeted sequence(s). **This statement is TRUE. Post-PCR detection techniques used routinely to differentiate methylated and unmethylated DNA include capillary electrophoretic separation, dideoxynucleotide sequencing, pyrosequencing, mass spectrometry, high performance liquid chromatography (HPLC), and array hybridization.**
b. Most methods for methylation analysis use sodium bisulfite to convert unmethylated cytosine to uracil. **This statement is TRUE. Methylated cytosines are protected from this process, resulting in different sequencing results in unmethylated and methylated samples.**
c. Bisulfite treatments are harsh, resulting in degradation of up to 85-95% of target sequences. **This statement is TRUE. Bisulfite conversion must be optimized to minimize DNA degradation.**
d. DNA treated with sodium bisulfite should be stored at 4°C to minimize further DNA degradation. **This statement is NOT TRUE. Ultra-low temperature storage conditions (-70°C and below) should be used to store sodium bisulfite-treated DNA to minimize further DNA degradation.**
e. Bisulfite conversion can be performed directly in tissue lysates to minimize DNA degradation. **This statement is TRUE. Alternatively, to decrease loss of DNA during bisulfite treatment, isolated DNA can be immobilized on nylon or in agarose.**

29. In methylation-specific PCR (MSP), sequence differences between the bisulfite-converted and unconverted cytosines are incorporated into the primer sequences used for amplification. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:266-278; DOI:10.2353/jmoldx.2009.080125; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. MSP provides a sensitive method for detecting minimal levels of methylated DNA in a sample. **This statement is TRUE. However, classical MSP is non-quantitative.**
b. Combining real-time PCR with MSP allows for quantitative assessment. **This statement is TRUE. MethyLight is one example of a quantitative MSP assay.**
c. Real-time SYBR-GREEN MSP requires specially designed MSP primers for quantitative analysis. **This statement is NOT TRUE. Real-time SYBR-GREEN MSP allows for quantitation using primers designed for non-quantitative MSP.**
d. Non-specific primer annealing may complicate quantitative MSP assay interpretation. **This statement is TRUE. Non-specific primer annealing will invalidate the readout.**
e. Gene methylation status should be correlated with expression levels or function. **This statement is TRUE. Gene expression can be confirmed using immunohistochemistry.**

30. Analysis of CpG methylation requires discrimination between methylated and unmethylated sequences. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:266-278; DOI:10.2353/jmoldx.2009.080125; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. In methylation-independent PCR, methylation differences are not incorporated into primers. **This statement is TRUE. Methylation differences are detected by another method after PCR, including combined bisulfite restriction analysis (COBRA), pyrosequencing, matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF), and HPLC.**
b. Methylation-sensitive restriction endonucleases can be used to differentiate between methylated and unmethylated CpG sites. **This statement is TRUE. This procedure does not require bisulfite treatment and therefore does not modify the DNA.**
c. Microarray-based techniques have been developed that allow for simultaneous testing of multiple CpG sites. **This statement is TRUE. These techniques work on both bisulfite-treated and untreated DNA.**
d. The number of CpG sites per sample and the estimated number of abnormal versus normal cells per sample help to determine which test to use. **This statement is TRUE. The amount and quality of the starting tissue, cells, or DNA should also be considered.**
e. For gene expression application, more sensitive methods are chosen over quantitative methods when homogenous samples are available. **This statement is NOT TRUE. When homogenous samples are available, quantitative methods are preferred because they can allow the use of cutoff-values that have been determined to correlate with actual gene silencing through a companion technique.**

31. CpG methylation analysis is important in detection, classification, and monitoring treatment response in various human cancers. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:266-278; DOI:10.2353/jmoldx.2009.080125; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Hereditary non-polyposis colon cancer (HNPCC) can be distinguished from other sporadic colorectal cancers with high microsatellite instability (MSI) by the absence of CpG methylation of the *MLH1* promoter. **This statement is TRUE. However, methylation of *MLH1* can also be seen as a second hit in individuals with germline *MLH1* mutations.**
b. In esophageal carcinoma, hypomethylation of *RIZ1, CRBP1*, and *APC* in Barrett's esophagus or low-grade dysplasia represent independent risk factors for progression to high-grade dysplasia or adenocarcinoma. **This statement is NOT TRUE. In esophageal carcinoma, hypermethylation of *P16*, *RUNX3,* and *HPP1* in Barrett's esophagus or low-grade dysplasia may represent independent risk factors for progression to high-grade dysplasia or adenocarcinoma.**
c. Concurrent methylation of multiple CpG islands is a hallmark for cholangiocarcinoma. **This statement is TRUE. Early detection of cholangiocarcinoma is important for proper treatment and patient survival.**
d. Aberrant promoter methylation of *DAPK1* and *CADM1* (IGSF4) occurs at a high frequency in high-grade squamous intraepithelial lesions, but is absent in low-grade squamous intraepithelial lesions. **This statement is TRUE. These molecular markers may be used to predict disease progression.**
e. Concurrent methylation of three or more CpG islands can differentiate low-grade papillary urothelial carcinoma lesions from benign/reactive urothelium in urine cytology samples. **This statement is TRUE. Although high grade urothelial carcinoma can be readily detected in urine cytology, cytologic detection of low-grade papillary urothelial carcinoma in urine cytology is challenging due to overlapping cytomorphologic features with benign reactive processes.**

32. CpG methylation is altered in some viral and bacterial infectious agents. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:266-278; DOI:10.2353/jmoldx.2009.080125; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Epstein-Barr virus may repress certain viral genes to avoid immune detection. **This statement is TRUE. Nuclear antigens EBNA 1-6 and LMP 1 and 2 are repressed in the host.**
b. JC virus T antigen expression is associated with widespread CpG methylation in colorectal cancer. **This statement is TRUE. This widespread CpG methylation is referred to as a CpG island methylator phenotype (CIMP).**
c. A novel targeted therapeutic strategy involves hypermethylation/repression of viral gene expression in virally-infected tumor cells. **This statement is NOT TRUE. The therapeutic strategy involved demethylation/activation of viral gene expression in virally-infected tumor cells to trigger immune recognition and destruction of these cells.**
d. Patients infected with *Helicobacter pylori* or that have inflammatory bowel disease exhibit hypermethylation of multiple CpG islands. **This statement is TRUE. These conditions lead to increased risk of cancer development.**
e. Demethylation agents may be useful in cancer chemoprevention in the gastric mucosa of patients with *H. pylori* infection. **This statement is TRUE. CpG methylation status may also be useful to clinically determine the risk of gastric cancer.**

33. Mutations in the hemoglobin beta (*HBB)* gene cause β-thallasemia. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:334-346; DOI:10.2353/moldx.2009.080151; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. To date, 56 point mutations in *HBB* have been reported. **This statement is NOT TRUE. To date, 739 point mutations in *HBB* have been reported, although each ethnic group studied has a limited number of common mutations.**
b. The c. 79G>A mutation is the most common mutation in Southeast Asia. **This statement is TRUE. This mutation is also known as CD26G>A or Hb E.**
c. In the Thai population, including all speakers of Thai languages, 30 variants account for more than 99.5% of all *HBB* mutant alleles. **This statement is TRUE. In the Thai population, approximately 40 *HBB* mutations have been identified.**
d. Restriction fragment length polymorphism (RFLP) analysis and reverse dot-blot (RDB) hybridization have both been used to genotype *HBB* mutations. **This statement is TRUE. Amplification refractory mutation system (ARMS), single strand conformation polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), and direct DNA sequencing have also been used in *HBB* screening.**
e. A high-throughput screening methodology has been developed for a number of *HBB* mutations. **This statement is TRUE. Allele-specific arrayed primer extension (AS-APEX), multiplex minisequencing, capillary electrophoresis (CE), denaturing high performance liquid chromatography (DHPLC), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) have all been used in high-throughput screening.**

34. Efficient and cost-effective screening methods are needed to genotype *HBB* mutations in the Thai population. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:334-346; DOI:10.2353/jmoldx.2009.080151; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Tetraplex PCR was used to amplify four fragments. **This statement is TRUE. The four fragments spanned all 30 mutations.**
b. Multiplex primer extension products were analyzed by MALDI-TOF MS. **This statement is TRUE. The multiplex primer extension products were first desalted by magnetic bead separation.**
c. The approach developed in the study reliably and unambiguously detected *HBB* mutations that were validated using a total of 162 randomly selected β-thalassemia samples. **This statement is TRUE. The validation samples were previously genotyped by DGGE, RFLP and/or direct sequencing techniques.**
d. Optimization of the primer extension reaction was achieved by varying combinations of extension primers while taking into account priority of *HBB*-mutation types. **This statement is TRUE. All primers in the set were determined to be compatible and individual masses were confirmed to be unique.**
e. Unequal signal intensities for heterozygous alleles resulted in frequent false-negative results, decreasing the sensitivity of the protocol. **This statement is NOT TRUE. Although unequal signal intensities for heterozygous alleles were frequently observed, false-negative results were not obtained from the protocol, resulting in 100% sensitivity. Unequal signal intensities for heterozygous alleles are acceptable unless the signals from both alleles are more than 10 fold different and the low signal no longer satisfies the signal-to-noise criterion for genotype determination.**

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http://jmd.amjpathol.org/
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ANSWERS for CME Questions # 35-42
35e, 36a, 37c, 38d, 39c, 40e, 41b, 42b

35. Polymorphisms in metabolic enzymes affect tamoxifen treatment outcome in breast cancer. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:381-389 DOI:10.2353/jmoldx.2009.090003; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Tamoxifen, which functions by disrupting estrogen signaling through the estrogen receptor (ER), requires extensive metabolism by cytochrome P450 (CYP) enzymes to be functional. **This statement is TRUE. Tamoxifen is metabolized by CYP enzymes to produce three metabolites of clinical interest: N-desmethyl-tamoxifen, 4-hydroxy-tamoxifen (4-OH-TAM), and endoxifen (4-hydroxy-N-desmethyl-tamoxifen), which is thought to be responsible for the majority of tamoxifen-associated anti-tumor effects.**

b. Tamoxifen therapy following chemotherapy reduces the 5-year recurrence rate by nearly 50% in patients with ER-positive breast cancer. **This statement is TRUE. Tamoxifen therapy also decreases the 15-year breast cancer mortality rate by one-third.**

c. Variants of CYP2D6 have different abilities to metabolize tamoxifen. **This statement is TRUE. There at least 70 CYP2D6 variants. They are categorized by enzymatic activity into poor, intermediate, extensive, and ultrarapid metabolizers.**

d. A patient's CYP2D6 genotype determines the success of tamoxifen treatment. **This statement is TRUE. Women with two or more fully functional alleles of CYP2D6 produce significantly higher levels of endoxifen, a pharmacogenetically regulated metabolite of tamoxifen, than women with one or more nonfunctional alleles.**

e. Randomized clinical trials indicate that aromatase inhibitors should be used instead of tamoxifen treatment in premenopausal patients with poor metabolizing variants of CYP2D6. **This statement is NOT TRUE. Whereas aromatase inhibitors have demonstrated superior efficacy and better overall safety than tamoxifen in postmenopausal patients, there are few data in premenopausal women.**

36. Success of breast cancer therapy can be predicted based on multi-gene analysis. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:381-389 DOI:10.2353/jmoldx.2009.090003; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Multi-gene analysis can identify genetic variations occurring in surrounding tumor stroma to predict response to tamoxifen. **This statement is NOT TRUE. Multi-gene analysis identifies genetic variations occurring specifically within malignant tumor cells to predict response to tamoxifen.**
b. There are currently two multi-gene assays that analyze genes involved in cell proliferation, as well as ER and HER-2 signaling pathways, in breast cancer. **This statement is TRUE. The ability of Onco*Type* DX (Genomic Health, Inc.) and MammaPrint (Agendia) to guide therapeutic selection for breast cancer is being investigated.**
c. Onco*Type* DX (Genomic Health, Inc.) and MammaPrint (Agendia) multi-gene assays require extraction of RNA from a tumor specimen to assess its gene expression profile. **This statement is TRUE. In contrast, CYP2D6 genotyping requires DNA extraction from blood to determine which CYP2D6 alleles a given patient carries.**
d. Based on the expression profile of 21 genes, the Onco*Type* DX multi-gene assay determines the 10-year risk for disease recurrence in patients with ER-positive, lymph node-negative tumors. **This statement is TRUE. An initial study demonstrated that Onco*Type* DX was useful in predicting the benefit from tamoxifen in patients with a low or intermediate, but not high, recurrence score.**
e. The MammaPrint multi-gene assay is FDA-approved to assess the likelihood of disease metastasis in breast cancer patients by categorizing them into low or high risk for breast cancer recurrence. **This statement is TRUE. The MammaPrint assay uses microarray-based technology to determine the expression profile of 70 genes.**

37. Irinotecan is an FDA-approved drug for first-line combined or second-line single-agent treatment of metastatic colorectal cancer; however, irinotecan toxicity is a major concern. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:381-389 DOI:10.2353/jmoldx.2009.090003; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Irinotecan is a topoisomerase I inhibitor that is both an active cytotoxic agent and a prodrug for its more potent metabolite SN-38. **This statement is TRUE. SN-38 is further metabolized by uridine diphosphate-glucuronyltransferase 1A1 (UGT1A1) to inactive SN-38-glucuronide (SN-38-G).**
b. Both irinotecan and SN-38 can be transformed by several CYPs, including CYP3A4, to minor inactive metabolites. **This statement is TRUE. Enzyme inhibition from irinotecan therapy is a concern for patients co-administered other CYP3A4 substrates.**
c. Although there is no immediate toxicity from irinotecan use, late-onset toxicity is characterized by diarrhea and other cholinergic symptoms. **This statement is NOT TRUE. Irinotecan can cause both immediate and delayed toxicity. Early adverse responses include diarrhea and other cholinergic symptoms, whereas late-onset toxicity is characterized by potentially life-threatening diarrhea, neutropenia, or both.**
d. Reduced expression or activity of uridine diphosphate-glucuronyltranferase 1A1 (UGT1A1) due to allelic variation prolongs exposure to SN-38, increasing risk of toxicity. **This statement is TRUE. Irinotecan toxicity has been associated with reduced UGT1A1 activity, and a promoter polymorphism with 7 thymine-adenine repeats that results in reduced UGT1A1 activity increases the risk of adverse events.**
e. There are several non-promoter missense mutations in UGT1A1 that may affect irinotecan toxicity. **This statement is TRUE. These missense mutations are found primarily in Asian populations. The most common is UGT1A1*6 (allele frequency ~20% in Asians), which results in alteration of arginine 71 to glutamine.**

38. Somatic tumor-specific mutations in pathways related to epidermal growth factor receptor (EGFR) signaling are associated with the therapeutic efficacy of EGFR inhibitors. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:381-389 DOI:10.2353/moldx.2009.090003; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. EGFR belongs to the family of tyrosine kinase receptors that also includes HER2/neu. **This statement is TRUE. In addition to trastuzumab (Herceptin), the successful anti-HER2/neu-directed breast cancer therapy, other medications targeting EGFR, including monoclonal antibodies (cetuximab, panitumumab), specific kinase inhibitors (gefitinib, erlotinib), and broader-spectrum kinase inhibitors (lapatinib, canertinib), are being explored.**
b. Several genes encoding key components of the downstream EGFR mitogen-activated protein kinase (MAPK) and phosphoinositol-3 kinase (PI3K) signaling cascades have been implicated in the success of EGFR-directed therapy. **This statement is TRUE. These signaling molecules include Raf, which stimulates MAPK; Akt, which enhances signaling downstream of PI3K; and Ras, which directly activates both MAPK and PI3K pathways.**
c. In the context of EGFR-directed therapy, alterations in *KRAS* and *EGFR* have very different consequences. **This statement is TRUE. Most activating mutations of EGFR enhance tumor susceptibility to therapeutic agents (inhibitors or antibodies) directed against the receptor, whereas KRAS gain-of-function bypasses EGFR entirely and therefore results in extremely poor response to directed therapies.**
d. Most small deletions or point mutations in the EGFR kinase region decrease susceptibility to specific EGFR inhibitors. **This statement is NOT TRUE. Most small deletions or point mutations in the EGFR kinase region increase susceptibility to specific EGFR inhibitors, although some somatic mutations (eg, Tyr790Met) have been found to confer resistance and can occur as secondary alterations after initially successful EGFR-directed therapy.**
e. Lung tumors with somatic alterations in *KRAS* show negligible response (<5% of patients) to EGFR-targeted agents. **This statement is TRUE. A similar situation exists in colorectal cancer, where the incidence of *KRAS* mutations is higher than in lung cancer (36% compared with 20%).**

39. Formalin-fixed, paraffin-embedded (FFPE) tissues are widely available; however, the use of these samples for gene profiling is limited. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:420-439 DOI:10.2353/jmoldx.2009.090041; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Messenger RNA (mRNA) is often extensively degraded in FFPE specimens. **This statement is TRUE. mRNA is often degraded to lengths of less than 300 base pairs.**
b. Formalin fixation results in covalent modification of RNA. **This statement is TRUE. Formalin fixation adds a mono-methylol group to RNA bases, cross-linking the nucleic acid to proteins. This cross-linking results in RNA strand breakage, making RNA extraction and quantitation difficult.**
c. MicroRNAs (miRNAs) are less stable than mRNAs in FFPE tissues. **This statement is NOT TRUE. MicroRNAs, which are endogenous ~22 nucleotide non-coding RNAs, are more stable than mRNAs in FFPE tissues.**
d. Small RNAs are stable in FFPE specimens for up to 10 years. **This statement is TRUE. miRNAs and small RNAs were purified from 10-year-old archived normal skin, cutaneous scalp melanoma, and sentinel lymph nodes (both negative and positive for metastasis) that had been excised from a 52-year-old man.**
e. Degraded ribosomal RNA molecules account for a large proportion of the RNA that can be extracted from FFPE samples. **This statement is TRUE. A 'poison primer' strategy (ie, primer silencing) was employed to block the amplification of ribosomal RNA, thus allowing enrichment of other small RNA species such as miRNA.**

40. miRNA screening may be beneficial in cancer profiling. Based on the referenced Technical Advance, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:420-439 DOI:10.2353/jmoldx.2009.090041; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. miRNAs can play important regulatory roles in cancer. **This statement is TRUE. miRNAs pair to the mRNAs of target genes and direct mRNA cleavage or repression of protein synthesis**.
b. Particular miRNAs may function as tumor suppressors or oncogenes. **This statement is TRUE. Abnormal expression levels of certain miRNAs may play a role in human cancer pathogenesis.**
c. Changes in miRNA levels may accompany dysregulated growth and apoptosis in some cancers. **This statement is TRUE. Reductions in expression of miR-15a and miR-16, let-7a, or miR-143 and miR-145 have been reported in chronic lymphocytic leukemia, lung cancer, and colorectal neoplasia, respectively.**
d. miRNA profiling may identify prognostic biomarkers. **This statement is TRUE. Prognostic miRNA biomarkers can be identified by using microarray hybridization or size-fractionated cDNA library sequencing.**
e. The authors of this paper developed a proteomics-based method to screen for miRNAs in cancerous tissues. **This statement is NOT TRUE. Ma et al developed a method to release high levels of high quality total RNA from various FFPE specimens, optimizing the method for small RNA discovery. Using the 'poison primer' strategy, they successfully sequenced 17 novel and 53 known miRNAs.**

41. Specific human papillomavirus (HPV) genotypes are associated with an increased risk of cervical cancer. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:439-445 DOI:10.2353/jmoldx.2009.080154; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Two HPV genotypes cause 70% of cervical cancers in the United States. **This statement is TRUE. HPV16 and HPV18 cause 70% of cervical cancers in the United States.**
b. HPV16 is more commonly associated with adenocarcinoma and adenosquamous carcinoma, whereas HPV18 is more commonly associated with squamous cell carcinoma. **This statement is NOT TRUE. HPV16 is more commonly associated with squamous cell carcinoma, whereas HPV18 is more commonly associated with adenocarcinoma and adenosquamous carcinoma.**
c. Infection with HPV types 16 and 18 has been associated with a higher risk for progression to carcinoma *in situ* (CIN) III or greater. **This statement is TRUE. HPV16 and HPV18 are also associated with progression of pre-cancerous cells.**
d. Persistent infection with the same HPV carcinogenic type may confer increased risk for high-grade lesions. **This statement is TRUE.**
e. Commercial genotyping methods may provide clinicians with information about infections with specific HPV types that may be helpful in certain clinical contexts. **This statement is TRUE. Presence of more carcinogenic phenotypes could lead to more aggressive therapy.**

42. Commercial methods for genotyping HPV are becoming increasingly available, but they require verification for use in the clinical laboratory. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:439-445 DOI:10.2353/jmoldx.2009.080154; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. The authors compared a commercially available HPV genotyping test, the INFINITI HPV-QUAD assay, with the FDA-approved digene Hybrid Capture 2 High-Risk HPV DNA Test (HC2) assay. **This statement is TRUE. Distinct methods used in the two tests can contribute to differences in both analytical sensitivity and specificity.**
b. There was an overall agreement of 25% between the HC2 and HPV-QUAD assays. **This statement is NOT TRUE. The overall agreement between the HC2 and HPV-QUAD assays was 83%.**
c. The HC2 assay uses solid phase antibody capture of RNA:DNA hybrids and amplified chemiluminescent signal detection. **This statement is TRUE. The use of multiple probes in the HC2 assay may provide additional sensitivity at the cost of reduced specificity and increased cross-reactivity, potentially causing false-positive results.**
d. The HPV-QUAD assay uses multiplex PCR followed by automated processing for primer extension, hybridization, and detection to genotype HPV. **This statement is TRUE. The authors evaluated the HPV-QUAD assay using liquid cervical cytology specimens.**
e. Denatured material from cervical collection brush specimens that remains after processing for the HC2 assay can be used for the HPV-QUAD genotyping assay. **This statement is TRUE. The use of residual denatured material for the HPV-QUAD assay could improve clinical laboratory workflow and overcome reflex testing concerns due to low quantities of samples.**

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ANSWERS for CME Questions # 43-50
43d, 44b, 45e, 46c, 47a, 48e, 49b, 50c

43. The World Health Organization (WHO) has recommended the augmentation of accurate and active diagnostic laboratory testing for Dengue virus (DENV). Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:537-542; DOI:10.2353/jmoldx.2009.080164; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. DENV is a vector-borne viral disease. **This statement is TRUE. DENV a major health risk in rural areas of endemic countries.**
b. Viral RNA extraction kits primary rely on two methods - liquid phase partition and silica-based nucleic acid adsorption chromatography. **This statement is TRUE. An example of liquid phase partition is TRIzol LS, and some examples of silica-based nucleic acid adsorption chromatography are the High Pure Viral RNA and QIAamp Viral RNA kits.**
c. The recovery of viral RNA by liquid phase partition and silica-based methods varies depending on the virus examined. **This statement is TRUE. This makes the choice of any particular method for the isolation of viral RNA difficult.**
d. The presence of high serum proteins severely affected the recovery of DENV RNA by the silica-based but not the liquid phase partition method. **This statement is NOT TRUE. The presence of high serum proteins severely affected the recovery of DENV RNA by the liquid phase partition, but not the silica-based method.**
e. DENV recovery was significantly improved by the addition of a co-precipitant. **This statement is TRUE. DENV recovery for the liquid phase partition method was significantly improved by the addition of a co-precipitant. In addition, reduction of sera proteins resulted in recoveries similar to that of the silica-based methods.**

44. Accurate molecular diagnosis of viral pathogens depends heavily on pre-analytic procedures. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:537-542; DOI:10.2353/jmoldx.2009.080164; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Storage and transport of patient samples and the stability of viral RNA in the samples have a major impact on the development and performance of any successful molecular diagnostic test. **This statement is TRUE. Methods of isolating high yield and quality viral RNA also have a major impact on the success of a molecular diagnostic test.**
b. Intact DENV was found to be stable in serum for up to 5 days at 25ºC. **This statement is NOT TRUE. Intact DENV was found to be stable in serum for up to 2 hours at 25ºC.**
c. Recovery of viral RNA from sera stored in lysis/binding buffer was possible for up to 5 days. **This statement is TRUE. The storage and transport conditions critically affect the quantification of viral nucleic acid from clinical samples.**
d. Repeated freeze-thaw cycles did not affect the recovery of DENV RNA. **This statement is TRUE. This result was unexpected since repeated freeze-thaw cycles generally have been shown to affect the integrity of cell-free RNA and viral RNA.**
e. The WHO-South-East Asia Regional Office (SEARO) recommends that DENV-infected patient sera be stored at -70ºC without thawing. **This statement is TRUE. The stability of DENV RNA after freeze-thaw was possibly due to the protective effects of serum protein on the stability of viral particles.**

45. Array comparative genomic hybridization (array CGH) is becoming integrated into the clinical diagnostic laboratory, where it is critical to have reliable and reproducible results. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:590-597; DOI:10.2353/jmoldx.2009.090009; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Clinical laboratories must define quality criteria for detecting copy number changes. **This statement is TRUE. Threshold values for copy number changes must also be set.**
b. Validation testing must be performed to demonstrate accuracy in array CGH testing and analysis. **This statement is TRUE. Diagnosticians should also go through proficiency testing.**
c. Variations in sample quality may affect array results. **This statement is TRUE. Clinical laboratories should implement quality control measures to attain accurate results with a high level of confidence.**
d. Clinical laboratories must strictly control environmental variables that may affect array CGH results. **This statement is TRUE. One such variable is ozone.**
e. Array CGH was performed in this study with a bacterial artificial chromosome (BAC) microarray that contained more than 6,000 BACs representing 3,000 loci with each locus represented by a minimum of two overlapping clones. **This statement is NOT TRUE. The version of the SignatureChip contains 4,685 BACs representing 1,543 loci with each locus represented by a minimum of three overlapping clones.**

46. Ozone has been shown to affect a class of cyanine dyes, predominately cyanine 5 (Cy5) and, to a lesser extent, cyanine 3 (Cy3), which are commonly used in array CGH. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:590-597; DOI:10.2353/jmoldx.2009.090009; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Ozone is formed when nitrous oxides and volatile organic compounds react in the presence of sunlight. **This statement is TRUE. Nitrous oxides and volatile organic compounds are emitted by motor vehicle exhaust, industrial emissions, gasoline vapors, chemical solvents, and natural sources.**
b. The seasonal emergence of ozone may affect reproducibility of array results. **This statement is TRUE. Ozone levels are higher during the summer months.**
c. Ozone levels are lower in urban and industrial areas, affecting result verification by different laboratories. **This statement is NOT TRUE. Ozone levels are higher in urban and industrial areas.**
d. Extremely low levels of ozone (5 to10 ppb) cause problems with array CGH. **This statement is TRUE. Ozone levels considered normal for environmental standards are well above those ranges demonstrating sensitivity of the dyes.**
e. As little as 10-30 seconds of ozone exposure can alter array CGH results. **This statement is TRUE. Manufacturers have developed more stable, high intensity dyes to overcome these difficulties.**

47. Exposure to ozone during microarray post-hybridization washes and scanning impacts array data. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11:590-597; DOI:10.2353/jmoldx.2009.090009; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. The red dyes in labeling kits were not discernibly affected by ozone. **This statement is NOT TRUE. The green dyes in labeling kits were not discernibly affected by ozone.**
b. The authors investigated the effects of ozone on microarray data by washing the array in variable ozone environments. **This statement is TRUE. In addition, the authors observed the effects of prolonged exposure to ozone on the microarray after washing in an ozone-free environment.**
c. Ozone effects varied for the three dye kits examined. **This statement is TRUE. The Alexa Fluor 3 and 5 dye set showed the least amount of damage over the 200 minute trial, whereas the Alexa Fluor 555 and 647 kit experienced the greatest amount of damage. The Cy3/Cy5 dye set performed equivalently with the Alexa Fluor 3 and 5 kit up to 120 minutes of exposure.**
d. Exposure to ozone during the post-hybridization washes has a considerable negative impact on array data. **This statement is TRUE. These results demonstrate the necessity to minimize ozone exposure when washing and drying the microarray.**
e. Washed microarrays produce the best results when immediately scanned. **This statement is TRUE. However, if a low-ozone environment is maintained, there is little compromise in the data if scanning is delayed.**

48. Hydatidiform moles (HMs) can be classified as complete hydatidiform moles (CHMs) or partial hydatidiform moles (PHMs). Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11: 598-605; DOI:10.2352/jmoldx.2009.090039; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. The risk of persistent gestational trophoblastic disease differs for CHMs, PHMs, and non-molar specimens (NMs). **This statement is TRUE. Clinical management of patients depends on accurate differentiation of CHMs, PHMs, and NMs.**
b. The diagnosis of HMs based solely on morphology suffers from poor interobserver reproducibility. **This statement is TRUE. Even among experienced pathologists, high inter- and intra-observer variability exists.**
c. Some ancillary techniques have been used to target genetic differences among CHMs, PHMs, and NMs, including cytogenetic analysis, determination of ploidy, fluorescent *in situ* hybridization, PCR amplification of short tandem repeat (STR) loci, and immunohistochemistry. **This statement is TRUE. Of these ancillary techniques, STR genotyping offers greater diagnostic discriminatory capability because CHMs, PHMs, and NMs can be specifically distinguished from one another based on identification of the parental source of polymorphic alleles and their ratios.**
d. Karyotyping and determining ploidy by flow cytometry are not sufficient to distinguish CHMs from NMs. **This statement is TRUE. These techniques cannot specifically discern the maternal and paternal chromosomal contributions in a specimen.**
e. CHMs (including early forms) can be distinguished from PHMs and NMs by immunohistochemical assessment of expression of the paternally imprinted *p21* gene. **This statement is NOT TRUE. CHMs (including early forms) can be distinguished from PHMs and NMs by immunohistochemical assessment of expression of the paternally imprinted *p57* gene. CHMs are characterized by lack of p57 expression in villous stromal cells and cytotrophoblast due to the lack of maternal DNA. However, p57 immunohistochemistry cannot distinguish PHMs from NMs since both retain expression of p57 due to the presence of maternal DNA.**

49. CHMs, PHMs, and NMs have distinct genetic features. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11: 598-605; DOI:10.2352/jmoldx.2009.090039; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. CHMs are most often diploid. **This statement is TRUE. PHMs, on the other hand, are triploid.**
b. Both chromosomal complements of CHMs are maternal in origin. **This statement is NOT TRUE. Both chromosomal complements of CHMs are paternal in origin (androgenetic diploidy).**
c. PHMs have one maternal chromosome complement and two paternal chromosome complements. **This statement is TRUE. This is called diandric triploidy.**
d. NMs typically have one maternal and one paternal chromosome complement (bi-parental diploidy). **This statement is TRUE. NMs are typically diploid.**
e. Some NMs can be triploid due to two maternal and one paternal chromosome complement (digynic triploidy). **This statement is TRUE. Triploid NMs also do not have morphological features of PHMs.**

50. STR genotyping can be utilized to better distinguish CHMs, PHMs, and NMs. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2009 11: 598-605; DOI:10.2352/jmoldx.2009.090039; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. STR genotyping distinguishes CHMs, PHMs, and NMs based on identification of the parental source of polymorphic alleles and their ratios. **This statement is TRUE. This analysis can discern androgenetic diploidy, diandric triploidy, and biparental diploidy.**
b. The largest problem with using STR genotyping to distinguish CHMs, PHMs, and NMs is contamination of villous tissue. **This statement is TRUE. Maternal tissue contamination of villous tissue often results in conflicting results from different STR loci. Education of both pathologists and technologists is the key to avoiding the problem of decidual contamination of the villous specimen.**
c. Use of STR data from 2 loci on one chromosome is sufficient for obtaining accurate results. **This statement is NOT TRUE. Use of STR data from 2 or more loci on separate chromosomes is essential for obtaining accurate results. In this article, the average number of informative and interpretable STR loci for CHM specimens was 5 (range 2 to 8).**
d. Rare examples of CHMs demonstrate androgenetic diploidy at most loci but retain one or a few maternal chromosomes (trisomy for these chromosomes). **This statement is TRUE. Analysis of only these loci would lead to misinterpretation as a triploid rather than a diploid specimen and a classification as PHM rather than CHM.**
e. Biparental CHM presents clinically, morphologically, and immunohistochemically (negative p57 result) as multiple CHMs, which appear to have a risk of persistent gestational trophoblastic disease similar to that of conventional CHM (uniparental androgenetic diploidy). **This statement is TRUE. Molecular genotypic analysis in such a case would result in biparental diploidy that could be misinterpreted as a non-molar gestation in the absence of correlation with morphologic features and p57 results.**

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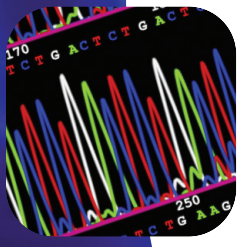

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