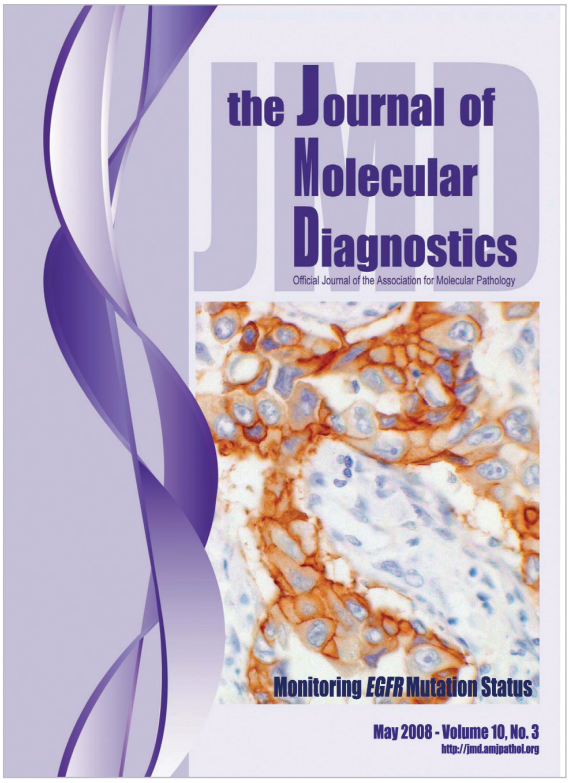

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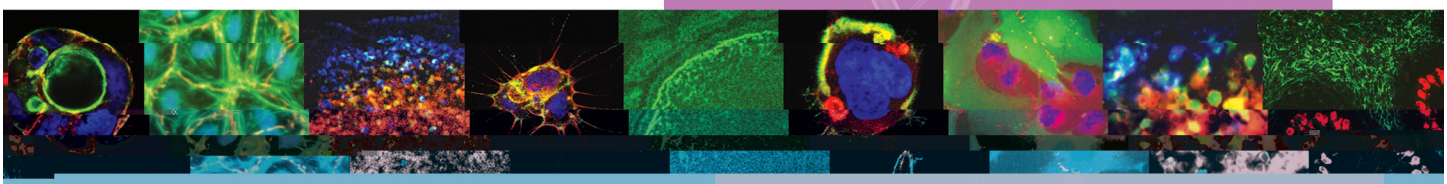
JMD CME Program in Molecular Diagnostics

Questions & Answers 2008



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JMD CME Program in Molecular Diagnostics 2008

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

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ANSWERS for CME Questions # 1-50

Issue No.	Month	Questions #	Answers
1	January	1-8	1b, 2a, 3d, 4b, 5d, 6a, 7c, 8e
2	March	9-18	9c, 10d, 11a, 12e, 13c, 14b, 15a, 16b, 17e, 18c
3	May	19-26	19b, 20a, 21c, 22e, 23a, 24d, 25a, 26d
4	July	27-34	27c, 28b, 29a, 30d, 31e, 32c, 33d, 34a
5	September	35-43	35d, 36b, 37e, 38a, 39c, 40e, 41c, 42c, 43a
6	November	44-50	44b, 45a, 46c, 47d, 48c, 49a, 50b

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

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

1. Fragile X syndrome is the most common inherited cause of mental retardation. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:2-12; most of the authors direct molecular genetic laboratories in academic institutions that offer clinical testing for fragile X on a fee-for-service basis; two of the authors received support from Celera (Alameda, CA); some of the authors are employed by Quest Diagnostics, Nichols Institute (Chantilly, VA), Myriad Genetic Laboratories, Inc. (Salt Lake City, UT), Amicus Therapeutics (Cranbury, NJ), and Sequenom, Inc (San Diego, CA).]

a. The incidence of Fragile X syndrome is higher in males than in females. **[This statement is TRUE: The incidence in males is 1 in 3500. The incidence in females is 1 in 8000.]**
b. In contrast to affected males, females with Fragile X syndrome exhibit severe retardation and behavioral abnormalities, including autism spectrum disorder. **[This statement is FALSE: Affected females have mild retardation. Males generally exhibit mental retardation and behavioral abnormalities.]**
c. Fragile X syndrome and *FMR1*-associated phenotypes are usually caused by expansion of a $(CGG)_n$ repeat sequence in the 5' untranslated region of the *FMR1* gene. **[This statement is TRUE: There are essentially four allelic forms of the repeat length: normal (5-44 repeats), gray zone or intermediate or borderline (45-54 repeats), premutation (55-200 repeats), and full mutation (>200 repeats). The boundaries between genotypic classes are currently defined by the American College of Medical Genetics.]**
d. Genetic testing for Fragile X mutations is important at all life stages, prenatal to adult. **[This statement is TRUE: Phenotypes associated with expansion of the *FMR1* gene include premature ovarian failure and fragile X-associated tremor/ataxia syndrome.]**
e. Some groups have suggested that *FMR1* testing should be offered to women diagnosed with premature ovarian failure and reproductive or fertility problems associated with elevated basal FSH levels as well as those with a low response to gonadotropin stimulation. **[This statement is TRUE.]**

2. Assays to analyze the triplet region of the *FMR1* gene are technically challenging. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:2-12; most of the authors direct molecular genetic laboratories in academic institutions that offer clinical testing for fragile X on a fee-for-service basis; two of the authors received support from Celera (Alameda, CA); some of the authors are employed by Quest Diagnostics, Nichols Institute (Chantilly, VA), Myriad Genetic Laboratories, Inc. (Salt Lake City, UT), Amicus Therapeutics (Cranbury, NJ), and Sequenom, Inc (San Diego, CA).]

a. The National Institutes of Standards and Technologies (NIST) has produced standard reference materials for Fragile X syndrome testing that are highly characterized and are produced in sufficient quantities to be utilized by most clinical laboratories in North America as a daily-use reference or quality control material. **[This statement is FALSE: The standard reference materials are produced in limited quantities and are intended for occasional use for assay validation or as calibration material but not as a daily-use reference or quality control material.]**
b. The high GC content of the $(CGG)_n$ repeat complicates conventional polymerase chain reaction (PCR) by reducing amplification efficiency. **[This statement is TRUE.]**

c. Genomic DNAs with known allele lengths are the most critical reference materials for laboratory-developed assay validation because they most closely resemble patient specimens. **[This statement is TRUE: Due to the clinical implications of the different size categories of premutations and all full mutations, the clinical laboratory must provide an accurate and precise assessment of *FMR1* $(CGG)_n$ allele length for patient samples.]**
d. Nine clinical laboratories measured the $(CGG)_n$ repeat size in DNA samples derived from 16 cell lines containing clinically relevant *FMR1* alleles in the normal and premutation range. **[This statement is TRUE: The laboratories used both in-house and a common, research-use-only platform to determine allele size.]**
e. Consensus was not achieved for all samples, including three DNA samples with the largest estimated allele sizes. **[This statement is TRUE.]**

3. Molecular classification of colorectal cancer is rapidly evolving. Based on the referenced Review article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:13-27; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. In general, as a primary discriminator in classification of colorectal cancers, emphasis should be placed on molecular classification based on global cellular events such as chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP). **[This statement is TRUE: In addition, single molecular events are also useful classifiers, particularly for predicting response to targeted therapies against specific molecules.]**
b. CIN is considered to promote carcinogenesis through loss of tumor suppressors and copy number gains of oncogenes. **[This statement is TRUE: CIN and MSI tend to be mutually exclusive in colorectal cancer.]**
c. CIN is commonly assessed by DNA ploidy analysis or loss of heterozygosity (LOH) analysis of microsatellite markers. **[This statement is TRUE.]**
d. Markers in the 1p region have been shown to be more sensitive for LOH analysis of CIN than markers in other chromosomal regions such as 2p, 3p, 5q, 8p, 17p and 18q. **[This statement is FALSE: Markers in the 18q region have been shown to be generally more sensitive than the other chromosomal regions.]**
e. Colorectal cancers that have multiple reciprocal translocations with little net changes in allele copy numbers or DNA content can be misclassified as CIN negative by copy number assays such as LOH or array-based comparative genomic hybridization. **[This statement is TRUE.]**

4. Transcriptional inactivation by cytosine methylation at promoter CpG islands of tumor suppressor genes is an important mechanism in human carcinogenesis. Based on the referenced Review article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:13-27; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. CIMP is a unique epigenetic phenotype in colorectal cancer. **[This statement is TRUE.]**
b. CIMP-high colorectal tumors have a distinct clinical, pathologic, and molecular profile, including associations with distal tumor location, male sex, poor differentiation, MSI, and high *TP53* mutation rates. **[This statement is FALSE: CIMP-high colorectal tumors are associated with proximal tumor location, female sex, poor differentiation, MSI, and high *BRAF* and low *TP53* mutation rates.]**
c. The method of assessment of CIMP involves different panels of CpG islands but is not yet standardized. **[This statement is TRUE: The CpG islands that are evaluated in current panels of methylation markers include *RUNX3*, *CACNA1G*, *IGF2*, *MLH1*, *NEUROG1*, *CRABP1*, *SOCS1* and *CDKN2A*.]**
d. The frequency of HNPCC/Lynch syndrome in the general population is estimated to be 1 to 3%. **[This statement is TRUE: However, a large population-based study has suggested that the frequency of MSI-H non-CIMP-high tumors is approximately 5%. It is thus likely that some MSI-H non-CIMP-high tumors arise through neither HNPCC/Lynch syndrome nor the CIMP-high pathway.]**
e. The absence of CIMP-high in MSI-H tumors increases the likelihood of HNPCC/Lynch syndrome, but it does not necessarily indicate HNPCC/Lynch syndrome. **[This statement is TRUE.]**

5. The use of appropriate extraction and amplification controls for acellular specimens such as cerebrospinal fluid, stool, and serum is not standardized in the clinical laboratory community and can lead to the reporting of false results. Based on the referenced Technical Advance article, select the ONE statement regarding internal controls that is NOT true: [See J Mol Diagn 2008 10:28-32; Attostar LLC (Medina, MN) provided free of charge reagents for initial evaluation; after an abstract related to this research study was accepted for presentation at a scientific meeting (by a Program Committee on which Attostar LLC played no role), Attostar LLC provided a travel grant to one author to attend the meeting; Attostar did not place any restrictions on the research activity or request pre-approval for submission of the abstract or the manuscript; No honoraria or consulting fees were provided, and none of the authors hold stock in the company.]

a. Extraction controls and amplification inhibitor checks for cellular specimens are most often accomplished by amplification of an internal human genomic target. **[This statement is TRUE: This approach is not feasible for acellular specimens, which may contain little or no amplifiable genomic material.]**
b. Ribosomal 16S or 18S RNA is a convenient control for reverse transcription-PCR. **[This statement is TRUE.]**
c. Packaging of plasmid-derived DNA in a bacteriophage can protect the DNA from nuclease degradation prior to extraction. **[This statement is TRUE: A disadvantage of packaging plasmid DNA in a bacteriophage is the additional time and expense.]**
d. An advantage of armored RNA is that inhibitors of reverse transcription and of PCR can be readily distinguished. **[This statement is FALSE: Armored RNA is nucleic acid packaged in bacteriophage coat proteins that protect the RNA from ribonucleases. Disadvantages of this technology are that it is proprietary and expensive, the reverse transcription process adds unnecessary time and expense to DNA-targeted assays, and inhibitors of reverse transcription and of PCR cannot be distinguished.]**
e. Specifically engineered λ phage DNA fragments have been generated to co-amplify with viral assays to detect the presence of PCR inhibitors, but these fragments are not used as extraction controls, they are assay specific, and construction of each synthetic phage is time consuming. **[This statement is TRUE.]**

6. A method to detect the presence of inhibitors, while simultaneously providing a control for extraction in acellular specimens, is needed to meet quality assurance standards recommended by clinical laboratory accrediting agencies. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:28-32; [See J Mol Diagn 2008 10:28-32; Attostar LLC (Medina, MN) provided free of charge reagents for initial evaluation; after an abstract related to this research study was accepted for presentation at a scientific meeting (by a Program Committee on which Attostar LLC played no role), Attostar LLC provided a travel grant to one author to attend the meeting; Attostar did not place any restrictions on the research activity or request pre-approval for submission of the abstract or the manuscript; No honoraria or consulting fees were provided, and none of the authors hold stock in the company.]

a. The T4 bacteriophage has a capsid that is composed of only 50 subunits. **[This statement is FALSE: The T4 bacteriophage has a capsid that is structurally complex, composed of more than 1,500 subunits.]**
b. Unaltered T4 bacteriophage is nonpathogenic, quantifiable, and inexpensive to produce. **[This statement is TRUE.]**
c. The T4 bacteriophage genome is roughly 169 kilobases long and contains approximately ten times more nucleotide base pairs than a typical plasmid DNA. **[This statement is TRUE.]**
d. The T4 bacteriophage capsid protects the encapsulated DNA from nucleases present in biological fluids. **[This statement is TRUE.]**
e. The T4 bacteriophage DNA detection assay was run simultaneously with the set of cycling parameters of in-house infectious disease tests, including those for cytomegalovirus, Epstein Barr virus, and herpes simplex virus, to test the implementation of T4 bacteriophage as a control to evaluate extraction efficiency and detect inhibitors of PCR in acellular specimens. **[This statement is TRUE.]**

7. Early detection of breast cancer improves survival rates and quality of life. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:93-101; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. A platform for multiplex detection of DNA methylation at multiple genomic sites was tested using DNA from formalin-fixed, paraffin-embedded clinical specimens. **[This statement is TRUE.]**
b. Infiltrating ductal carcinoma (IDC) was defined as malignant mammary epithelial cells invading stroma. **[This statement is TRUE: Samples of well, moderately and poorly differentiated IDC were examined.]**
c. Atypical ductal hyperplasia (ADH) was defined as lesions between 2 mm and 4 mm in size and having all of the characteristics of low-grade ductal carcinoma *in situ* (DCIS) or lesions larger than 4 mm having only some characteristics of DCIS. **[This statement is FALSE: ADH was defined as lesions less than 2 mm in size having all the characteristics of low-grade DCIS or lesions larger than 2 mm having only some characteristics of DCIS.]**
d. Samples of ADH were from core biopsies, whereas other specimens were from gross sections of surgically removed tissues. **[This statement is TRUE: Gross sections were not enriched for tumor cells and contained variable amounts of stromal and tumor cells, whereas core biopsies of ADH were more homogeneous.]**
e. Specific methylation signatures were identified for ADH, DCIS, and IDC. **[This statement is TRUE: The results suggest that informative cancer-specific methylation signatures can be detected in heterogeneous tissue specimens.]**

8. Denaturing high performance liquid chromatography (DHPLC) profiling has been suggested as a method to allow the correlation of a characteristic chromatographic profile with a specific sequence alteration. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:102-108; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. The screening for mitochondrial tRNALeu(UUR) A3243G mutation in the diabetic population using blood samples is problematic because blood may contain low levels of the A3243G mutation, which can be as low as 3% in some patients with maternally inherited diabetes mellitus and deafness. **[This statement is TRUE: Although muscle biopsy has become a routine procedure in the diagnostic testing for mitochondrial diseases, muscle samples are not easily obtained from diabetic patients.]**
b. Published protocols were used to attempt to detect the mitochondrial tRNALeu(UUR) A3243G mutation in blood DNA obtained from the mother of a patient affected with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). **[This statement is TRUE.]**
c. Site-directed mutagenesis was used to generate a panel of 5 mitochondrial mutations to explore whether mutations lying in the tRNALeu(UUR) region of the mitochonodrial genome can be identified by their chromatographic patterns. **[This statement is TRUE: A characteristic profile was found in only 1 of 5 mutants tested whereas 4 other mutants showed identical chromatographic profiles.]**
d. Heteroduplex levels in the purified heteroduplex fraction of the mutation standards fell short of the theoretically predicted level of 50%. **[This statement is TRUE: The underlying reason for reduced mutant DNA species in the purified heteroduplex fractions was that the homoduplex DNA species coeluted with the heteroduplex DNA species.]**
e. The degree of heteroplasmy for some mitochondrial DNA mutations varies considerably among tissues with urine epithelial cells and muscle specimens reported to carry lower levels of mutation than peripheral blood lymphocytes. **[This statement is FALSE: Muscle specimens and urine epithelial cells carry higher levels of mutation than peripheral blood lymphocytes.]**

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

ANSWERS for CME Questions # 9-18
9c, 10d, 11a, 12e, 13c, 14b, 15a, 16b, 17e, 18c

9. Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy that afflicts over 200,000 individuals worldwide every year. Based on the referenced Review Article of the molecular genetics of PDAC, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:111-122; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. PDAS is the fourth most common cause of cancer-related deaths in the United States. **[This statement is TRUE: There are more than 33,000 fatalities annually in the US.]**
b. PDAC constitutes over 90% of pancreatic cancers in humans. **[This statement is TRUE.]**
c. The most common numerical changes observed in pancreatic cancer are losses on chromosomes 7 and 20, as well as gains on chromosomes 12 and 13. **[This statement is FALSE: The most common numerical changes observed in pancreatic cancer are losses on chromosomes 6, 12, 13, and 18, as well as gains on chromosomes 7 and 20.]**
d. Frequent chromosomal breaks and rearrangements in PDAC occur in regions involving 1p, 1q, 3p, 6q, 7q, 11p, 17p, and 19q. **[This statement is TRUE.]**
e. Allelotyping studies have revealed allelic losses that commonly involve chromosomal regions 9p, 17p, and 18q. **[This statement is TRUE: These regions cover the tumor suppressor genes *CDKN2A*, *TP53*, and *DPC4*/*SMAD4*/*MADH4*, respectively.]**

10. A variety of genomic DNA abnormalities are found in PDAC, including chromosomal aberrations, copy number changes, and activating mutations of oncogenes. Based on the referenced Review Article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:111-122; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Results from studies examining copy number aberrations should be interpreted with caution. **[This statement is TRUE: Copy numbers determined in tumor cells are not necessarily identical throughout the whole tumor and might vary over time, in response to chemotherapeutic agents or in metastatic foci as compared to primary tumors.]**
b. Major drawbacks of conventional comparative genomic hybridization (CGH) on metaphase spreads include low resolution and frequent difficulties in precisely mapping the regions of genomic amplifications or losses. **[This statement is TRUE.]**
c. Allelotype analysis of microdissected pancreatic intraepithelial neoplasia (PanIN) samples revealed loss of heterozygosity in chromosomal regions also found in pancreatic cancer. **[This statement is TRUE: Regions of allelic loss in PanIN include 9p, 17p, and 18q.]**
d. In PDAC, the activating point mutation within the *KRAS* oncogene affects codon 14. **[This statement is FALSE: Activating point mutations within the *KRAS* oncogene are present in over 80% of pancreatic cancers and most commonly affect codon 12. Activating mutations in codons 13 and 61 are also found.]**
e. Rare pancreatic cancers with wild-type *KRAS* usually harbor mutations of *BRAF*. **[This statement is TRUE: Both KRAS and BRAF function in activating the same Ras/Raf/MAP kinase signaling pathway.]**

11. In addition to intragenic mutations and allelic loss, silencing of tumor suppressor genes through epigenetic mechanisms is a frequent finding in many cancers. Based on the referenced Review Article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:111-122; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Aberrant DNA methylation is not found in PanIN lesions and appears to be specific for advanced disease. **[This statement is FALSE: Aberrant DNA methylation occurs in PanIN-2 and -3 lesions. Detection of aberrantly methylated DNA in clinical samples as a strategy for early detection of pancreatic cancer is an area of active research.]**
b. Epigenetic silencing in PDAC often affects genes that function as tumor suppressors or are involved in key homeostatic pathways. **[This statement is TRUE.]**
c. Promoter hypermethylation of human Hedgehog interacting protein was found in the majority of examined pancreatic cell lines and primary tumor samples, potentially contributing to increased Hedgehog signaling observed in pancreatic cancers. **[This statement is TRUE.]**
d. Hedgehog inhibition with cyclopamine has been found to increase cytotoxic effects of paclitaxel treatment and radiation on pancreatic cancer cells *in vitro* and to inhibit growth of pancreatic cancer xenografts and metastases *in vivo*. **[This statement is TRUE: Development of new, clinically applicable Hedgehog inhibitors is currently being pursued.]**
e. Promoter hypomethylation is also exploited by pancreatic cancer cells. **[This statement is TRUE: Some genes, including *maspin*, *S100P*, *mesothelin*, *prostate stem cell antigen*, and *claudin-4*, can be overexpressed due to promoter hypomethylation.]**

12. Laser capture microdissection (LCM) is widely used for genome and transcriptome profiling of tumor tissues. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:129-134; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Formalin-fixed, paraffin-embedded (FFPE) tissues are not ideal starting materials for genome-wide molecular profiling. **[This statement is TRUE: DNA and RNA extracted from FFPE tissues are highly fragmented because of the formalin-induced cross-linking.]**
b. After incubation of LCM-captured cells for 30 minutes with the guanidium-based extraction buffer RLT Plus, less DNA was extracted as compared to the QIAamp DNA purification method. **[This statement is TRUE.]**
c. RNA integrity was not affected by extending the time of extraction with guanidium-based extraction buffer at room temperature. **[This statement is TRUE.]**
d. There was severe RNA degradation with the guanidium-based extraction buffer when the extraction temperature was raised to 42°C or 55°C. **[This statement is TRUE.]**
e. DNA was severely degraded when the guanidium-based extraction was conducted at temperatures of 42°C or 55°C. **[This statement is FALSE: DNA integrity was less affected than RNA integrity.]**

13. Viruses of the genus *Flavivirus* are responsible for severe encephalitic, hemorrhagic, hepatic, and febrile illnesses in humans and other vertebrates. Based on the referenced article, select the ONE statement regarding internal controls that is NOT true: [See J Mol Diagn 2008 10:135-141; some of the authors are co-inventors on a pending U.S. Patent, "Microbial Identification Based on the Overall Composition of Characteristic Oligonucleotides," which has been exclusively licensed to BioTex, Inc (Houston, TX).]

a. Flaviviruses are single-stranded RNA viruses. **[This statement is TRUE.]**
b. It is estimated that over 100 million cases of dengue occur annually worldwide. **[This statement is TRUE: The four serotypes of the mosquito-borne dengue virus can cause dengue hemorrhagic fever and dengue shock syndrome.]**
c. A protective dengue virus vaccine is currently available. **[This statement is FALSE: There is no protective vaccine or specific treatment available for dengue virus infection.]**
d. Typing of dengue virus is important in treatment because infection by a new serotype in a patient previously infected by one of the other serotypes is associated with an increased risk of developing dengue hemorrhagic fever and/or dengue shock syndrome. **[This statement is TRUE.]**
e. Typing of dengue virus is important for distinguishing endemic strains from new outbreak strains so that new outbreaks can be readily contained. **[This statement is TRUE.]**

14. Development of rapid and specific molecular diagnostics for flaviviruses is an important global health challenge. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:135-141]*

a. The current reference method for identifying and typing flaviviruses is isolation of the virus in cell culture followed by immunofluorescence typing. **[This statement is TRUE: This procedure is time-consuming and requires significant expertise and cell culture facilities that may not be readily available in developing countries where dengue is endemic.]**
b. Tests such as hemagglutination inhibition, IgG-ELISA, and MAC-ELISA, are specific, are easy to use, can accommodate a large number of samples, and can easily distinguish dengue at the serotype level. **[This statement is FALSE: These alternative serological tests cannot easily distinguish dengue from other flaviviruses at the serotype level and are likely to generate false-positives.]**
c. The authors derived "almost-universal" primer pairs for flaviviruses in general and for all four serotypes of dengue from the literature. **[This statement is TRUE: The coverage of each primer pair was evaluated against large sequence datasets.]**
d. DEN2 and DEN3 can be readily resolved by base-specific cleavage masses derived from the NS5 region. **[This statement is TRUE.]**
e. RNase T_1 is an endoribonuclease that is highly specific for cleavage after G residues; however, incomplete digestion products yield final masses other than those predicted by complete sequence cleavage after every G. **[This statement is TRUE: The intermediate product of endoribonuclease treatment, a 2'-3' cyclic phosphate intermediate, produces a mass 17 Da less than that of the final product.]**

15. Standard IUPAC base degeneracies are often used to develop degenerate forward and reverse primers for amplification. Based on the referenced article, select the ONE statement defining IUPAC nomenclature that is NOT true: [See J Mol Diagn 2008 10:135-141]*

a. D = A or G. **[This statement is FALSE: D = A, G, or T. R = A or G.]**
b. H = A, T, or C. **[This statement is TRUE.]**
c. Y = C or T. **[This statement is TRUE.]**
d. M = A or C. **[This statement is TRUE.]**
e. S = G or C. **[This statement is TRUE.]**

16. Anti-epidermal growth factor receptor (EGFR) antibody therapy is currently available for the treatment of some cancers. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:160-168]

a. The clinical experience with cetuximab in the treatment of colon carcinoma patients revealed that both EGFR-expressing tumors and tumors without detectable EGFR expression by standard immunohistochemistry had clinical responses. **[This statement is TRUE.]**
b. Gefitinib and erlotinib are small-molecule inhibitors of the tyrosine kinase domain of the EGFR that had severe side effects in colon cancer and lung cancer patients, resulting in premature termination of clinical trials. **[This statement is FALSE: Gefatinib and erlotinib had an objective response rate of 9 to 19% and mild side effects. There was rapid and dramatic tumor shrinkage in some patients.]**
c. Characteristics associated with increased response of lung cancers to gefitinib and erlotinib include nonsmoking history, adenocarcinoma histology, Asian race, and female gender. **[This statement is TRUE.]**
d. Gefitinib-treated patients carrying *EGFR* amplification (detected by fluorescent *in situ* hybridization) to high polysomy had a statistically significant improvement in response, time to progression, and survival compared with patients with no or low genomic gain for EGFR. **[This statement is TRUE.]**
e. In the current study, some non-small cell lung cancer (NSCLC) patients with immunohistochemistry-negative but *EGFR*-mutant NSCLC tumors had complete responses to erlotinib treatment. **[This statement is TRUE: Detectable protein biomarker overexpression is not a prerequisite for the presence of increased gene copy number or activating mutations and responsiveness to EGFR inhibitors in patients with lung adenocarcinomas.]**

17. Imatinib is the recommended first line therapy for patients with chronic myeloid leukemia (CML). Based on the referenced Consultations in Molecular Diagnostics article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:177-180]

a. More than 80% of CML patients treated with imatinib will achieve a complete cytogenetic response (CCR). **[This statement is TRUE.]**
b. After CCR is achieved, molecular response measured by reverse transcription-polymerase chain reaction quantification (RQ-PCR) is typically used to continue therapeutic monitoring and to detect loss of response at an early stage before overt relapse occurs. **[This statement is TRUE.]**
c. Most CCR patients on standard imatinib therapy will have residual disease detectable by RQ-PCR. **[This statement is TRUE.]**
d. The most common mechanism for a subsequent loss of response is acquired imatinib resistance due to the development of mutations in the BCR-ABL kinase domain that interfere with optimal drug-target interactions. **[This statement is TRUE: Kinase domain mutations have been detected in the majority of patients with acquired imatinib resistance.]**
e. Single nucleotide point mutations in the kinase domain are rarely reported in cases of imatinib resistance. **[This statement is FALSE: Almost all mutations in the kinase domain are single nucleotide point mutations. Insertions and deletions are rarely reported.]**

18. The spectrum of BCR-ABL kinase domain mutations discovered in CML patients with imatinib resistance is quite heterogeneous. Based on the referenced Consultations in Molecular Diagnostics article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:177-180]

a. In three CML patients undergoing kinase inhibitor therapy, direct DNA sequencing of BCR-ABL RT-PCR products revealed that the same 35 nucleotides from ABL intron 8 had been inserted at the normal exon 8-9 splice junction. **[This statement is TRUE.]**
b. The insertion created a premature translational stop codon after 10 intron-encoded amino acids, resulting in truncation of 653 C-terminal amino acids, which included part of the kinase domain and the entire “last exon” region. **[This statement is TRUE.]**
c. Consensus splice donor and acceptor sequences were not detected flanking the 35-bp intronic sequence, excluding alternative splicing as the likely mutational mechanism. **[This statement is FALSE: The 35-bp insert was flanked by excellent consensus splice donor and acceptor sequences, suggesting alternative splicing as the likely mutational mechanism. However, no specific nucleotide sequence substitutions were detected in the expressed cDNA sequences.]**
d. In the patient population studied, the estimated prevalence of the exon 8/9 insertion/truncation mutation was approximately 1.7% among patients with suspected drug resistance. **[This statement is TRUE.]**
e. The ages of the three patients with the unusual exon 8/9 insertion/truncation mutation varied between 15 and 60 years. **[This statement is TRUE.]**

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ANSWERS for CME Questions # 19-26
19b, 20a, 21c, 22e, 23a, 24d, 25a, 26d

19. More than 50 emerging and reemerging pathogens have been identified over the past four decades. Based on the referenced Review of the molecular diagnostics of emerging pathogens, select the ONE match between infectious agent and resulting disease that is NOT true: [See J Mol Diagn 2008 10:185-197; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. *Bartonella henselae* causes cat scratch disease. **[This statement is TRUE: *B. henselae* also causes bacillary angiomatosis.]**
b. Sin Nombre virus causes bacillary angiomatosis. **[This statement is FALSE: Sin Nombre virus causes hantaviral pulmonary syndrome.]**
c. *Anaplasma phagocytophilium* causes human granulocytotropic anaplasmosis. **[This statement is TRUE.]**
d. Nipah virus causes encephalitis. **[This statement is TRUE: Nipah virus is an RNA virus of family *Paramyxoviridae*, genus *Heniparus* and is found in Malaysia, Singapore, and Bangladesh. Bats are reservoirs of the virus.]**
e. Marburg virus causes hemorrhagic fever. **[This statement is TRUE: Marburg virus causes a severe form of hemorrhagic fever, characterized by prominent microvascular leakage and hemorrhages.]**

20. Molecular assays have played critical roles in the discovery, surveillance, and clinical laboratory diagnosis of newly emerging and reemerging viral respiratory infections in recent years. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:185-197; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Influenza A has 10 H and 12 N subtypes, of which only subtypes H1, H2, and N2 have stable lineages in avian and human populations. **[This statement is FALSE: Influenza A has 15 H and 9 N subtypes, all of which occur in birds. Subtypes H1, H2, H3, N1, and N2 have stable lineages in the human population.]**
b. H and N antigenic variants determined by point mutations cause seasonal influenza epidemics, whereas new antigenic H and N subtypes introduced by reassortment of viral genes cause pandemics. **[This statement is TRUE: Avian influenza A virus crossed the species barrier to infect humans during the 1918 pandemic and in recent infections by avian H5N1 influenza virus.]**
c. Influenza A has no pathognomonic symptoms, and diagnosis of influenza A based on clinical signs is correct in only two-thirds of patients. **[This statement is TRUE: Sensitive, rapid laboratory tests are required to guide antiviral use. Influenza A virus replication can be detected in respiratory secretions, and the viral load remains high for 72 hours. Reverse transcription-polymerase chain reaction (RT-PCR) can provide a definitive diagnosis especially early in the infection, along with antigen detection.]**
d. Several consensus and subtype-specific molecular assays have been developed to detect the four human coronaviruses (HCoV); however, the genetic variability of HCoVs makes the detection of all circulating strains technically challenging. **[This statement is TRUE: Subtypes 229E, OC43, NL63, and HKU1 cause mild upper respiratory illness with sore throat, rhinorrhea, cough, and fever and account for one-third of common cold cases.]**
e. Human bocavirus (HBoV) is a newly discovered human parvovirus with a worldwide distribution and a high prevalence of up to 18%. **[This statement is TRUE: HBoV was first identified by random PCR/cloning techniques on respiratory samples. HBoV is found concurrently with other infectious agents in up to 80% of cases. Because similar symptoms can be produced by the co-infecting agents, it has been questioned whether HBoV causes respiratory tract disease or is only a coincidental finding.]**

21. Food-borne outbreaks of *Cyclospora cayetanensis* have occurred around the world. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:185-197; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. *C. cayetanensis* was first described as a human pathogen in 1994. **[This statement is TRUE.]**
b. Infection occurs via the fecal-oral route by ingestion of contaminated water or produce. **[This statement is TRUE: The disease is characterized by watery diarrhea, cramping, and bloating, as well as weight loss if prolonged. If not treated the diarrhea persists for weeks to months.]**
c. Human-to-human infection is very likely because of the short sporulation time after shedding in feces. **[This statement is FALSE: Human-to-human infection is made less likely because the sporulation time is at least 7 days after shedding in feces.]**
d. *C. cayetanensis* oocytes are similar to those of *Cryptosporidium* but are twice the diameter. **[This statement is TRUE: Oocytes can be visualized using modified acid-fast stains or a modified safanin technique. They are also visualized under ultraviolet light due to autofluorescence. When compared to ultraviolet detection, the sensitivity of the acid-fast technique is about 78%]**
e. PCR detection of *C. cayetanensis* in human feces, produce, and water employs primers that target the internal transcribed spacer (ITR) region, with analytical sensitivity of 10 oocytes/gm of feces. **[This statement is TRUE.]**

22. Molecular assays have played important roles in the identification, surveillance, and clinical diagnosis of emerging viral hepatitides. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:185-197; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Hepatitis E virus (HEV) is a non-enveloped single-stranded, positive-sense RNA virus that is transmitted enterically. **[This statement is TRUE: HEV is common in tropical and subtropical regions.]**
b. No chronic sequelae develop after infection with HEV, but fulminant hepatitis occurs especially in pregnant women. **[This statement is TRUE: The disease is most severe during the third trimester, and mortality approaches 20%.]**
c. The genomic organization of HEV is close to rubella virus. **[This statement is TRUE: However, morphologically the closest related viruses are the *Caliciviridae* family.]**
d. Hepatitis delta virus (HDV) requires hepatitis B virus (HBV) to complete its life cycle in the eukaryotic host cell. **[This statement is TRUE: HDV replication depends on hepatitis B surface antigen (HBsAg) for packaging of the HDV genome.]**
e. All patients co-infected with HBV and HDV develop cirrhosis and die of end-stage liver disease. **[This statement is FALSE: When both HBV and HDV are present, the risk of severe disease is four-fold greater than in patients infected with HBV alone. The spectrum of disease when both viruses are present ranges from asymptomatic to end-stage liver disease (cirrhosis). In addition, superinfected patients are at risk for developing acute fulminant hepatitis.]**

23. Acute myelogenous leukemia (AML) is a heterogeneous disease clinically, molecularly, and cytogenetically. Based on the referenced Commentary and related articles, select the ONE statement regarding new molecular diagnostic tests for cytogenetically normal AML that is NOT true: [See J Mol Diagn 2008 10:198-202; J Mol Diagn 2008 10:212-216; and J Mol Diagn 2008 10:236-241; the authors of the referenced articles did not disclose any potential conflicts of interest.]

a. Approximately 25% of adult AML and 10% of pediatric AML cases do not harbor genetic alterations that can be detected by conventional cytogenetics. **[This statement is FALSE: Approximately 40 to 50% of AML cases have normal karyotypes with variable prognoses. Approximately 25% of pediatric cases are cytogenetically normal.]**
b. In the 2001 World Health Organization (WHO) classification of AML, four recurrent cytogenetic abnormalities have been selected to define specific diagnostic entities, with favorable prognoses found in AMLs that harbor gene fusions resulting from t(8;21), t(15;17) or inv(16). **[This statement is TRUE: WHO will release an update in 2008 that includes three additional genetically-defined AML categories with t(6;9), inv(3), and t(1;22).]**
c. The prognosis of tumors with 11q23 translocations depends on the chromosomal partner. **[This statement is TRUE: Tumors with 11q23 translocations tend to be more aggressive, with a poor or intermediate prognosis depending on the chromosomal partner.]**
d. The use of targeted therapies such as all-*trans*-retinoic acid is essentially restricted to those AMLs with t(15;17) gene fusions. **[This statement is TRUE.]**
e. In approximately 5% of cases, bone marrow may be superior to peripheral blood in detecting clonal abnormalities. **[This statement is TRUE: Methodology is also a factor in the variability in the frequency of cytogenetically normal AMLs. A higher yield of clonal abnormalities can sometimes be found by culturing cells for 24 or 48 hours than by direct preparation.]**

24. Many recurrent genetic abnormalities have been identified in AML that are important in both tumorigenesis and clinical outcome. Based on the referenced Commentary and related articles, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:198-202; J Mol Diagn 2008 10:212-216; and J Mol Diagn 2008 10:236-241; the authors of the referenced articles did not disclose any potential conflicts of interest.]

a. Partial tandem duplications of *MLL* are associated with a poor prognosis. **[This statement is TRUE: *MLL* is also disrupted in 11q23 translocations.]**
b. Internal tandem duplication within *FLT3* has a significantly adverse effect on clinical outcome. **[This statement is TRUE: The defect leads to ligand-independent activation of this receptor tyrosine kinase.]**
c. Translocations such as t(8;21) and t(15;17) that disrupt transcription factors (*RUNX1* and *RARA*, respectively) in a dominant-negative fashion lead to a block in normal myeloid differentiation (so-called class 2 mutations), but experimentally AML manifests only when translocations are combined with an additional mutation having a positive effect on proliferation (a so-called class 1 mutation). **[This statement is TRUE: In patients, the combination of the two types of mutations typically has prognostically adverse connotations.]**
d. Mutations in *CEBPA* correlate with a poor prognosis. **[This statement is FALSE: Mutations in *CEBPA* correlate with a favorable prognosis.]**
e. The most frequently identified mutated gene in cytogenetically normal AML is nucleophosmin (*NPM1*). **[This statement is TRUE: Nucleophosmin-1 (NPM1) is a nucleocytoplasmic shuttling protein that plays a key role in promotion of ribosome biogenesis, maintenance of genomic stability, regulation of transcription and modulation of tumor-suppressor transcription factors.]**

25. The *NPM1* gene is one of the most frequent targets of genetic alterations in hematopoietic tumors such as lymphomas and acute leukemia. Based on the referenced Commentary and related articles, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:198-202; J Mol Diagn 2008 10:212-216; and J Mol Diagn 2008 10:236-241; the authors of the referenced articles did not disclose any potential conflicts of interest.]

a. Point mutations in *NPM1* in AML all involve exon 6. **[This statement is FALSE: All point mutations in *NPM1* in AML involve exon 12. They were originally identified due to the resulting mislocalization of the mutant protein to the cytoplasm. *NPM1* mutations alter tryptophan residues required for proper nucleolar localization and create a putative nuclear export signal at the C-terminus of the protein.]**
b. Over 40 different types of mutations in the *NPM1* locus have been described so far, which result in the formation of different mutant proteins. **[This statement is TRUE: Most mutations consist of insertions and deletions of short nucleotide stretches (4 or 10 base pairs) in *NPM1* exon 12 that lead to an open reading frame shift. As a result, different protein variants containing novel C-terminus portions are formed.]**
c. Mutation A (NPM1-mutA) consists of a duplication of a TCTG tetranucleotide at position 956 to 959 of the reference sequence (GenBank accession number NM_002520). **[This statement is TRUE: NPM1-mutA is the most common NPM1 mutation type, accounting for over 75% of cases.]**
d. The clinical impact of *NPM1* mutations is affected by the mutational status of the *FLT3* gene. **[This statement is TRUE: Combining the status of *NPM1* and *FLT3* allows for stratification into 3 prognostic groups: good (*FLT3-ITD*$^-$/*NPM1*$^+$), intermediate (*FLT3-ITD*$^-$/*NPM1*$^-$ or *FLT3-ITD*$^+$/*NPM1*$^+$) and poor (*FLT3-ITD*$^+$/*NPM1*$^-$).]**
e. *NPM1* mutations are stable over the course of the disease and may serve as an ideal target for minimal residual disease assessment. **[This statement is TRUE.]**

26. The analysis of *NPM1* mutational status is now recommended for inclusion in the routine genetic characterization of AML. Based on the referenced Commentary and related articles, select the ONE statement regarding new molecular diagnostic tests for cytogenetically normal AML that is NOT true: [See J Mol Diagn 2008 10:198-202; J Mol Diagn 2008 10:212-216; and J Mol Diagn 2008 10:236-241; the authors of the referenced articles did not disclose any potential conflicts of interest.]

a. A non-quantitative, genomic DNA-based PCR assay for detecting all known *NPM1* mutations has been developed that utilizes intronic primers to avoid the amplification of known pseudogenes. **[This statement is TRUE: The assay utilizes a polymerase with editing capabilities to reliably distinguish between wild-type and mutant alleles with small length nucleotide insertions.]**
b. The relatively low analytic sensitivity of the genomic-DNA-based assay is not a limitation at initial diagnosis but it diminishes its utility for minimal residual disease detection. **[This statement is TRUE: The assay can detect mutations when the leukemic cells represent 5% of the population. At the time of diagnosis there are, by definition, at least 20% blasts.]**
c. The genomic-DNA-based assay can be used on paraffin-embedded tissue. **[This statement is TRUE.]**
d. A methodology utilizing an RNA template that uses allele-specifiic oligonucleotide (ASO) primers detects over 40 *NPM1* mutations. **[This statement is FALSE: The RT-PCR assay detects only the tetranucleotide TCTG insertion in exon 12 present in NPM1-mutA.]**
e. The RNA ASO-based assay can detect mutant clones that represent as little as 0.001% of the population. **[This statement is TRUE.]**

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

27. Epstein-Barr virus (EBV) causes infectious mononucleosis and is also associated with a wide variety of malignancies and disorders. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:279-292; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. The prevalence of EBV-related cancers is estimated to affect up to 1% of the world's population. **[This statement is TRUE: EBV is associated with a wide variety of malignancies and disorders, including Hodgkin and non-Hodgkin lymphomas, post-transplant lymphoproliferative disorder, nasopharyngeal carcinoma, and gastric carcinoma.]**
b. High-risk individuals may benefit from screening tests that predict impending progression so that preemptive measures may be taken even before disease is clinically evident. **[This statement is TRUE: Improvements in EBV-directed therapy highlight the importance of laboratory detection and the potential for targeting viral gene products or their downstream pathways that drive cell proliferation, inhibit apoptosis, or evade immune response.]**
c. EBV infects up to 10% of the adult population. **[This statement is FALSE: EBV infects nearly all adults, in which the viral genome is retained for life in a small fraction of B lymphocytes. Any biopsy tissue may contain B lymphocytes and therefore may harbor amplifiable EBV DNA.]**
d. Healthy carriers seem to harbor EBV almost exclusively in B lymphocytes. **[This statement is TRUE: Healthy carriers have approximately 1 to 50 infected cells per million leukocytes, consistent with an average EBV viral load in whole blood of about 7 copies (range 1 to 30 copies) of EBV DNA per million leukocytes.]**
e. In healthy carriers, cell-free body fluids such as serum or plasma contain negligible amounts of EBV DNA. **[This statement is TRUE: This suggests that EBV is detectable in serum or plasma only in association with reactivated infection or EBV-related disease.]**

28. EBV is capable of infecting a wide spectrum of cell types. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:279-292; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. EBV is capable of infecting B lymphocytes, squamous and glandular epithelial cells, myoepithelial cells, smooth muscle cells, T cells, NK cells, plasma cells, and follicular dendritic cells. **[This statement is TRUE.]**
b. Children with Bruton's agammaglobulinemia are particularly susceptible to infection with EBV and harbor a very high viral load in their B lymphocytes compared to most patients with infectious mononucleosis. **[This statement is FALSE: EBV infection does not take hold in children with Bruton's agammaglobulinemia, which is a rare genetic disorder in which B cells are absent.]**
c. When a cell is infected with EBV, the double-stranded viral DNA circularizes to form an episome that may then replicate to produce 1 to 50 clonal copies of the EBV genome. **[This statement is TRUE: These clonal episomes are passed along to cellular progeny. Although EBV DNA typically persists as an episome, in some instances it recombines with the human genome to create one or more chromosomal integrations.]**

d. The number of tandem repeat sequences found at the ends of the EBV genome varies from virion to virion. **[This statement is TRUE: The novelty of each virion relies on the number of tandem repeat sequences (up to 20) found at the ends of the linear EBV genome.]**
e. Latent infection is characterized by limited expression of viral proteins, such as EBV nuclear antigen 1 (EBNA1), which avoids immune recognition and destruction. **[This statement is TRUE: EBNA1 functions to propagate the viral genome to daughter cells upon cell division. It is unable to elicit an effective cytotoxic immune response, partially explaining why EBV is never eliminated from the body.]**

29. Serologic tests are often used to confirm infection and document remote infection. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:279-292; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. The heterophile antibody test (colloquially called the "Monospot" test) is no longer widely used to diagnose patients with infectious mononucleosis-like symptoms. **[This statement is FALSE: The Monospot test is the most widely used serologic assay. The original heterophile test was based upon the discovery that serum or plasma from patients with infectious mononucleosis could agglutinate horse or sheep erythrocytes. Modern variants of the test detect serum-mediated agglutination of latex beads coated by bovine heterophile antigens.]**
b. Mononucleosis-like symptoms include fever, sore throat, lymphadenopathy, hepatosplenomegaly, malaise, and headache. **[This statement is TRUE.]**
c. EBV-related cancer is typically associated with high serologic titers against early antigen (EA) and IgG viral capsid antigen (VCA) with low EBNA titer. **[This statement is TRUE: Results should be interpreted with caution since similar patterns are possible in autoimmune disease and other reactive conditions.]**
d. Serology is not reliable when the immune system is dysfunctional, such as in AIDS or allogeneic transplant patients. **[This statement is TRUE.]**
e. Nasopharyngeal cancer patients often have high IgA titers against lytic EBV proteins. **[This statement is TRUE: This is consistent with the origin of the cancer on the mucosal surface of the nasopharynx. A panel of serologic and molecular tests on serum or plasma from high-risk populations can screen for nasopharyngeal cancer, assess prognosis, and monitor disease status over time.]**

30. EBV-encoded RNAs (*EBERs*) are reliably expressed in virtually all latently infected cells in both benign and malignant lesions. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:279-292; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. *EBER in situ* hybridization is the assay of choice in clinical laboratories for defining a lesion as EBV-related. **[This statement is TRUE.]**
b. *EBER1* and *EBER2* are non-polyadenylated RNA transcripts that are abundantly expressed in latently infected cells. **[This statement is TRUE: *EBERs* are not translated into protein, but they function to inhibit interferon-mediated antiviral effects and apoptosis.]**
c. Oral hairy leukoplakia is an exceptional lesion in which *EBER* is downregulated. **[This statement is TRUE: In oral hairy leukoplakia, lytic proteins BZLF1 and BMRF1 are localized to ballooned cells in mid-layers of the hyperplastic stratified squamous epithelium.]**
d. Nearly all keratinizing nasopharyngeal carcinomas are EBV-related as demonstrated by *EBER in situ* hybridization. **[This statement is FALSE: Nearly all undifferentiated nasopharyngeal carcinomas (NPCs) are EBV-related whereas a lesser proportion of keratinizing NPCs harbor EBV.]**
e. In Western nations, anaplastic large cell lymphoma is virtually never *EBER*-positive. **[This statement is TRUE: Anaplastic large cell lymphoma is a histologic mimic of Hodgkin lymphoma. Hodgkin and non-Hodgkin lymphomas generally lack *EBER*-expressing small lymphocytes whereas the larger tumor cells may be uniformly *EBER*-positive. This feature can be helpful in resolving a benign *versus* malignant differential diagnosis since enlarged lymph nodes from infectious mononucleosis patients harbor variable numbers of *EBER*-expressing small to large lymphocytes.]**

31. Automation is becoming important to improve efficiency and standardization in the clinical laboratory. Based on the referenced Technical Advance article that compares automated nucleic acid extraction methods with manual extraction, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:311-316; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Three extraction methods were evaluated for their ability to afford nucleic acid for optimal polymerase chain reaction (PCR) amplification. **[This statement is TRUE: The five organisms/matrices used for PCR testing were *Bordatella pertussis*/bronchoalveolar lavage, herpes simplex virus II/cerebrospinal fluid, coscackievirus A9/cerebrospinal fluid, BK virus/plasma, and *Mycoplasma pneumoniae*/endotracheal tube samples.]**

b. The easyMAG system (bioMerieux, Durham, North Carolina) was found to be a user-friendly instrument, requiring 280 total steps to produce nucleic acid from 15 samples, compared to 534 for the manual extraction. **[This statement is TRUE: The easyMAG system may be used in laboratories that require moderate to high throughput.]**
c. Both the EZ1 (Qiagen, Valencia, California) and easyMAG systems considerably decreased hands-on-time compared to the manual method of extraction, saving between 29 and 47 minutes per batch, depending on the method. **[This statement is TRUE: The easyMAG system performed better than the EZ1 system in this regard. In a set of 15 samples, the EZ1 Instrument required almost twice as much hands-on-time as the easyMAG.]**
d. The setup of the easyMAG system does not change greatly when extracting specimens of different matrices and targets, while the EZ1 uses different program cards and reagent kits depending on target and matrix. **[This statement is TRUE: Single piece flow decreases errors and hands-on-time so that technologist time can be utilized effectively.]**
e. Including reagents, disposables, and technical time to determine the cost of extraction, the easyMAG system is the least expensive of the extraction methods. **[This statement is FALSE: The easyMAG system is the most expensive of the extraction methods. The cost to extract a sample using the easyMAG was $12.95 in this study, almost twice that of either the EZ1 system or manual extraction.]**

32. Sensitive detection of tumor-specific point mutations is of interest in both the early detection of cancer and the monitoring of treatment at a molecular level. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:325-331; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Somatic mutations in the K-*ras* gene are present in at least 80% of pancreatic cancers and 35 to 50% of colorectal cancers. **[This statement is TRUE.]**
b. Peptide nucleic acid (PNA) is a synthetic DNA analogue in which the ribose/phosphate backbone of the DNA in PNA is replaced by N-(2-aminoethyl)-glycine units linked by peptide bonds. **[This statement is TRUE.]**
c. PNA oligomers bind so strongly to complementary DNA that one single mismatch in the PNA oligomer will not destabilize the complex between oligomer and DNA target. **[This statement is FALSE: One single mismatch will severely destabilize the complex, typically lowering the melting temperature by 13-20^{o}C.]**
d. The sensitivity of PNA clamp PCR to detect K-*ras* mutations was limited by the low fidelity of Taq DNA polymerase. **[This statement is TRUE: Replication errors introduced by Taq polymerase in the PNA-binding site were amplified during PCR due to the resulting mismatches between PNA and DNA. The reported error rate of Taq DNA polymerase on different templates varies from 2x10^{-4} to 1x10^{-5} errors per nucleotide.]**
e. The sensitivity of the PNA clamp assay to detect K-*ras* mutations increased approximately 10-fold when Phusion HS DNA polymerase was used. **[This statement is TRUE.]**

33. Cystic fibrosis (CF) is a common and serious condition with autosomal recessive inheritance. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:368-375; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) on chromosome 7q31. **[This statement is TRUE: CF is characterized by malfunction of chloride ion channels and or transport pathway regulation.]**
b. *CFTR* is primarily expressed in the apical membrane of exocrine epithelial cells. **[This statement is TRUE.]**
c. The CF phenotype is variable, ranging from mild with limited manifestations to severe with rapid deterioration and death within the first year of life. **[This statement is TRUE: Classic CF is characterized by failure to thrive, recurrent bacterial endobronchitis, progressive decline of lung function, exocrine pancreatic dysfunction, and infertility in males. The severity of clinical manifestations depends on the set of *CFTR* mutations, modifying genes, and other variables.]**
d. Large deletions and insertions are the most frequently reported sequence variants that have been reported to the Cystic Fibrosis Mutation Database. **[This statement is FALSE: To date, more than 1500 sequence variants have been reported to the Cystic Fibrosis Mutation Database (http://www.genet.sickkids.on.ca/cftr/) and include point mutations, deletions, insertions, frameshift mutations and splice-site variants that lead to protein truncation. The overall frequency of large rearrangements is probably underestimated due to the testing methods commonly used but is likely to account for several percent of all affected alleles.]**
e. The range and frequency of individual *CFTR* mutations varies among different populations, ethnic backgrounds, and geographic locations. **[This statement is TRUE: The incidence of CF is approximately 1/3000 individuals and is highest in Caucasians and Ashkenazi Jews.]**

34. In Hispanics, the CF mutation spectrum remains relatively poorly defined. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:368-375; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. The CF carrier frequency in Hispanics of ~1 in 3000 is significantly lower than that in Caucasians. **[This statement is FALSE: The carrier frequency is ~1 in 46, compared to a carrier frequency in Caucasians of ~1 in 29. The Hispanic population is not homogeneous and is typically defined more by geographic origins in various regions of Southern Europe or the Americas, rather than by one specific genetic background.]**
b. Deletions involving exons 2-3, exons 17a-18, exon 20, exons 22-23, exons 22-24, and exon 24 have been described in Hispanic individuals. **[This statement is TRUE: Although the combined results from several studies do not yet allow an accurate estimate of CFTR exon deletion frequencies in Hispanic chromosomes, several of these deletions (exon 20, exons 2-3, and exons 22-23) have been recurring and may indicate relatively high frequencies in Hispanic CF patients.]**
c. The 935delA mutation is a relatively common mutation in Hispanics. **[This statement is TRUE.]**
d. Small deletion mutations are especially likely to interfere with probe hybridization in multiplex ligation-dependent probe amplification (MLPA) analysis. **[This statement is TRUE: For any condition in which MLPA is considered as an early step, either because it is relatively economical compared to sequencing methods or because deletions and duplications are a common type of mutation in that disorder, sequence verification near the probe ligation site should be performed as an initial check of data validity.]**
e. Apparent exon deletions by MLPA may indicate the presence of both large deletions and point mutations. **[This statement is TRUE: This has important implications for pan-ethnic MLPA testing in CF and other genetic conditions.]**

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ANSWERS for CME Questions # 35-43
35d, 36b, 37e, 38a, 39c, 40e, 41c, 42c, 43a

35. Immunoglobulin (*IG*) gene rearrangement analysis is one of the more commonly performed molecular hematopathology assays. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:396-410; the author of the referenced article did not disclose any potential conflicts of interest.]

a. Physiologic gene rearrangements refer to the normal intragenic shuffling of segments of antigen receptor genes, namely *IG* genes and T-cell receptor (*TCR*) genes in B and T cells, respectively. **[This statement is TRUE: Physiologic gene rearrangements represent a major mechanism in the generation of immunologic diversity.]**
b. The *BCR-ABL1* fusion generated as a consequence of the t(9;22) in chronic myelogenous leukemia is an example of an intergenic translocation that causes disruption of genes, with the subsequent fusion of portions of the disrupted genes, resulting in the generation of a novel, pathologic chimeric gene and ultimately chimeric oncoprotein. **[This statement is TRUE: The *BCR-ABL1* fusion is also found in a subset of adult precursor B-cell acute lymphoblastic leukemias.]**
c. Translocation involving the *LMO2* gene in T-cell acute lymphoblastic leukemia is an example of an intergenic translocation involving the removal of negative regulatory elements. **[This statement is TRUE: Heightened expression of an intact gene involved in an intergenic translocation (such as is found with the overexpression of *BCL2* as a consequence of being juxtaposed with the immunoglobulin heavy chain gene (*IGH@*) in the t(14;18) associated with follicular lymphoma) is not always due to the apposition of enhancers or promoters of contextually highly expressed genes.]**
d. There is a normal hierarchy of *IG* gene rearrangements, with light chains rearranging before *IGH@* in normal B-cell ontogeny. **[This statement is FALSE: *IGH@* rearranges before the light chains in normal B-cell ontogeny.]**
e. Finding an immunoglobulin light chain rearrangement is more likely to reflect the commitment to bona fide B-cell rather than T-cell lineage. **[This statement is TRUE: However, this is not absolute.]**

36. Analysis of antigen receptor gene rearrangements (ARGRs) can be considered for initial diagnosis and for subsequent minimal residual disease (MRD) studies. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:396-410; the author of the referenced article did not disclose any potential conflicts of interest.]

a. The inability to assess lymphoid tissue architecture may compromise the ability to render a diagnosis. **[This statement is TRUE: With the use of less-invasive diagnostic procedures such as fine needle aspiration and thin needle core biopsies, pathologists are being called upon to render diagnoses on smaller amounts of tissue. As such, an important facet in the evaluation of lymphoid tissue is the ability to assess architecture.]**

b. For initial diagnosis in specialized hematopathology centers, even when the tissue is optimal for microscopic and immunophenotypic analysis, the vast majority of ARGR cases require clonality testing. **[This statement is FALSE: In the diagnostic setting, when evaluating lymphoid tissue microscopically, and when the tissue is optimal both qualitatively and quantitatively, it is usually straightforward to distinguish neoplastic from reactive disorders and it is not necessary to perform ARGR analysis for diagnostic purposes. ARGR studies may be helpful in a minor subset of cases in which the histomorphologic features and immunophenotypic findings are equivocal. The frequency with which such cases require clonality testing by ARGR analyses is estimated to be ~30% of cases in laboratories with limited specialization in hematopathology and ~10% of cases in laboratories at specialized hematopathology centers.]**
c. In the initial phase of chemotherapy, the ability to reduce the level of acute lymphoblastic leukemia (ALL) below a certain threshold is considered an extremely favorable prognostic variable. **[This statement is TRUE: The ability to achieve a 4 log reduction in the level of disease from diagnosis is considered to be an extremely favorable variable in ALL. Cases in which there is less than a 2 log reduction fare more poorly and may be considered for alternative therapeutic modalities.]**
d. MRD testing can be applied to stem cell products that are to be used for autologous transplantation to ensure that the reinfused material is free of contaminating tumor. **[This statement is TRUE.]**
e. For MRD measurements to be clinically relevant, sensitivities need to be achieved on the order of 0.01%. **[This statement is TRUE: Such levels cannot be attained with primers used in diagnostic testing. MRD analysis must be performed by quantitative (typically real-time) PCR, using primers and/or probes that are specific for the *IGH*@ gene rearrangement present in the B-cell neoplasm that is being monitored.]**

37. In the early bone marrow phase of B-cell development prior to antigen exposure, the phenomenon of V(D)J recombination is the basis of clinical analysis of *IG* gene rearrangements. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:396-410; the author of the referenced article did not disclose any potential conflicts of interest.]

a. Recombination signal sequences (RSSs) flank V, D, and J segments and provide binding sites for the primary enzyme complex that mediates the initial DNA cleavage and synapsis required for legitimate recombination. **[This statement is TRUE: RSSs consist of conserved heptamers and nonamers, separated by nonconserved spacers, typically of 12 or 23 nucleotides.]**
b. With regard to the *IGH*@ locus, DJ rearrangement occurs first and, only after this is completed, is V to DJ rearrangement able to occur. **[This statement is TRUE: There is a hierarchy both in the order of *IG* gene rearrangements at the different loci and within the *IGH*@ locus.]**
c. The enzyme terminal deoxynucleotidyl transferase (TdT) generates junctional diversity by mediating the random deletion and addition of nucleotides at the sites of V to D, as well as D to J, fusion. **[This statement is TRUE: TdT creates N regions between V and D, and D and J.]**
d. The recombinase activating genes *RAG1*/*RAG2* and the exonuclease activity of the DNA repair machinery contribute to junctional diversity by the inclusion of pallindromic (P) nucleotides. **[This statement is TRUE: Based on the number of functional *IGH*@ V, D, and J segments, there are ~10^4 possible recombination *IGH*@ recombination events.]**
e. The most distal (3') complementarity determining region (CDR) of the fully rearranged *IGH*@ gene (VNDNJ) is the most homogeneous of the three CDRs since it is encoded in the germline and is not affected by either recombination or the action of TdT. **[This statement is FALSE: The most distal CDR (CDR3) is the most heterogeneous since it is affected by both recombinatorial and junctional diversity mechanisms. By contrast, the more proximal (5') CDR1 and CDR2 are encoded in the germline and are not affected by either recombination or the action of TdT.]**

38. The immunoglobulin molecules generated by VDJ recombination in the bone marrow are of low affinity and must acquire higher affinity for pathogenic antigens to be functionally effective. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:396-410; the author of the referenced article did not disclose any potential conflicts of interest.]

a. Class switch recombination (CSR) typically occurs in the dark zone of the germinal center, which is characterized by smaller centrocytes. **[This statement is FALSE: The dark zone is characterized by proliferating larger centroblasts, and it is predominantly here that somatic hypermutation (SHM) happens. CSR typically occurs in the light zone, which is dominated by smaller centrocytes.]**
b. Activation-induced cytosine deaminase (AID) mediates both CSR and somatic hypermutation (SHM). **[This statement is TRUE: Expression of AID appears to be restricted to germinal center B cells.]**

c. Mutations in *IG* genes are predominantly point mutations, although insertions and deletions may also occur. **[This statement is TRUE: Transition mutations (pyrimidine to pyrimidine or purine to purine) are approximately twice as common as transversions.]**
d. Mutations in *IG* genes occur approximately six orders of magnitude more often than spontaneous mutations at other loci. **[This statement is TRUE.]**
e. Although SHM is somewhat influenced by the primary sequence of the DNA, it is essentially a random phenomenon. **[This statement is TRUE: SHM is targeted toward certain hotspot motifs such as DGYW (D= adenosine, guanosine (G) or thymidine; Y= cytidine or thymidine; W= adenosine or thymidine), indicating that it is somewhat influenced by the primary sequence of the DNA. Furthermore, SHM is targeted to a ~1-2 kb region downstream of the transcription start site (after which SHM exponentially decreases), affecting certain CDRs more than others.]**

39. MicroRNAs (miRNAs) are emerging as potential markers in molecular diagnostics, particularly in the field of cancer diagnostics. Based on the referenced Commentary and related Technical Advances article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:411-414 and J Mol Diagn 2008 10:415-423; five of the authors of the referenced Technical Advance article are employees of Asuragen, Inc., and one author has an affiliation with Luminex Corp; none of the authors disclosed any potential conflicts of interest with companies whose products were evaluated in this article.]

a. MiRNAs are important gene regulators that have the capacity to down-regulate gene expression of target genes through translation inhibition and promotion of mRNA degradation. **[This statement is TRUE: Promotion of mRNA degradation is mediated by specific target site binding to the 3' untranslated region of target genes.]**
b. According to the most recent version of miRBase, over 600 different mature miRNA sequences have been identified in humans. **[This statement is TRUE: Version v.11.0 of miRBase identifies 678 different mature miRNA sequences.]**
c. Many miRNAs are highly specific in their expression in specific tissues and cell types; however, this specificity is rarely retained in the corresponding tumor tissues. **[This statement is FALSE: The tissue and cell type specificity are often retained in the corresponding tumor tissues.]**
d. An miRNA classifier consisting of 48 miRNAs was found to predict tissue origin with an overall accuracy of 89%. **[This statement is TRUE: The overall accuracy of the miRNA classifier was evaluated in an independent blinded test set of 83 samples using an algorithm in which one to five specific miRNAs determined the decision at each node of a binary decision tree.]**
e. Cell-type-specific miRNA signatures correlate with mRNA expression patterns. **[This statement is TRUE: Highly expressed mRNAs tend to lack binding sites of highly expressed miRNAs in the same tissues. Levels of mRNAs targeted by certain miRNAs tend to be lower in tissues in which those miRNAs are present at high levels.]**

40. The application of miRNAs for molecular diagnostic purposes is dependent on the development of methods for their accurate and high-throughput quantification. Based on the referenced Commentary and related Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:411-414 and J Mol Diagn 2008 10:415-423; five of the authors of the referenced Technical Advance article are employees of Asuragen, Inc., and one author has an affiliation with Luminex Corp; none of the authors disclosed any potential conflicts of interest with companies whose products were evaluated in this article.]

a. The real-time RT-PCR for quantitative measurement of miRNAs is similar to the standard real-time RT-PCR for the detection of mRNA, except that the former makes use of a stem-loop reverse transcription primer for the initiation of cDNA templates. **[This statement is TRUE: This method is easy to use, is highly sensitive, and has a broad dynamic range.]**
b. The Invader assay directly detects specific RNA molecules using an isothermal amplification process with a fluorescent read-out. **[This statement is TRUE: After some modifications for miRNA quantification, the sensitivity, specificity, dynamic range and ease of use of the Invader assay appear to be comparable to real-time RT-PCR.]**
c. The method of choice for the simultaneous analysis of hundreds of different miRNAs is global expression profiling, which is most often performed on glass slide microarrays. **[This statement is TRUE.]**
d. Global expression profiling of miRNAs can be performed using bead-based flow cytometry. **[This statement is TRUE.]**
e. The use of locked nucleic acid (LNA)-modified probes in miRNA microarrays has not been successful due to decreased specificity. **[This statement is FALSE: The use of LNA-modified probes has enhanced the specificity and sensitivity of miRNA microarrays.]**

41. Large-scale analysis of miRNA microarray expression signatures can be accomplished by accessing archived formalin-fixed, paraffin-embedded (FFPE) human specimens. Based on the referenced Commentary and related Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:411-414 and J Mol Diagn 2008 10:415-423; five of the authors of the referenced Technical Advance article are employees of Asuragen, Inc., and one author has an affiliation with Luminex Corp; none of the authors disclosed any potential conflicts of interest with companies whose products were evaluated in this article.]

a. MiRNA has been shown by RT-PCR to be a superior analyte compared to mRNA when FFPE materials are used since miRNAs are small and therefore less subject to RNA degradation. **[This statement is TRUE: The formalin fixation process allows for permanent preservation of the architecture of tissues in optimal histological condition and easy long-term storage.]**
b. Independent of the tissue type, miRNA expression profiles in tissues fixed in formalin for 6 hours are nearly identical to those fixed for 24 hours. **[This statement is TRUE.]**
c. Irrespective of their level of expression, 100% of the miRNAs expressed in frozen tissues are detected in FFPE samples. **[This statement is FALSE: While 100% of the miRNAs highly expressed in frozen tissues are detected in FFPE samples, the detection of miRNAs expressed at medium or low levels in frozen samples is gradually lost over time in FFPE samples, and can be as low as 30% for low abundance miRNAs in 11 year old FFPE samples.]**
d. Failure to capture a larger percentage of miRNAs in FFPE samples may be a consequence of a lower miRNA fraction recovery or excessive RNA modification during FFPE tissue storage. **[This statement is TRUE.]**
e. A gradual increase in the probe signal intensity of miR-494 and -513 was detected in aging myometrium tissue blocks, peaking at 460- and 161-fold in the 11-year-old samples compared to frozen tissue. **[This statement is TRUE: This unexpected increase in abundance could be indicative of degradation of precursor miRNA or mRNA species that produced fragments that were nonspecifically labeled and hybridized to miR-494 and -513 probes on the array.]**

42. The fragile X syndrome is the most common inherited disorder associated with mental retardation. Based on the referenced Consultations in Molecular Diagnostics article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:469-474; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. The fragile X syndrome in males is characterized by large ears, speech delay, autistic behavior, macroorchidism, and mental retardation. **[This statement is TRUE: The estimated prevalence in males is 1 in 4,000.]**
b. The estimated prevalence of fragile X syndrome in females is 1 in 8,000. **[This statement is TRUE.]**
c. Fragile X female patients uniformly exhibit severe learning and behavioral defects. **[This statement is FALSE: Females with a full mutation exhibit a wide spectrum of phenotypes, ranging from no detectable learning or behavioral deficits to effects as severe as those in male fragile X patients with a full mutation.]**
d. Over 99% of fragile X cases have been associated with an expansion of a segment of CGG repeats in the 5' untranslated region of the *FMR1* gene. **[This statement is TRUE: In addition to the most common expansion of CGG repeats, a few mutations, such as deletions and point mutations, have been identified in fragile X patients.]**
e. Full mutations with a large CGG expansion in the 5' untranslated region of *FMR1* are associated with inhibition of transcription of *FMR1*, causing deficiency or absence of the fragile X mental retardation protein. **[This statement is TRUE: There are 4 allelic forms of the CGG repeat lengths: normal (5-44 repeats), intermediate (gray zone, 45-54 repeats), premutation (55-200 repeats), and full mutation (>200 to 230 repeats).]**

43. Diagnostic testing for the fragile X syndrome is designed to detect the most common mutation. Based on the referenced Consultations in Molecular Diagnostics article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:469-474; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. In normal females, the 5.2-kb band on a Southern blot assay for fragile X syndrome represents the active, unmethylated X chromosome. **[This statement is FALSE: In normal females, the 2.8-kb band represents the active, unmethylated X chromosome, whereas the 5.2-kb band represents the inactive, methylated X chromosome.]**

b. Methylated full mutations are not detectable by polymerase chain reaction (PCR) and are often shown as bands larger than 5.8 kb on a Southern blot. **[This statement is TRUE.]**
c. High resolution chromosome analysis of the 7-year-old patient showed a normal female karyotype, and molecular cytogenetic analysis using an LSI Prader-Willi/Angelman region probe showed no deletion or duplication in metaphase and interphase cells. **[This statement is TRUE: This analysis excluded the possibility that her autistic feature was due to 15q duplication.]**
d. A 10.9-kb band was detected on the standard Southern blot of the proband but not her parents, while a Southern blot using only PstI digestion showed the predicted, normal-sized bands in the patient and her parents. **[This statement is TRUE: No extra band was observed in the index family, excluding the possibility that the 10.9-kb band is due to a large CGG expansion at the 5' untranslated region of *FMR1*.]**
e. The proband inherited a mutation in the EcoRI recognition site of *FMR1* at the 11114 position from her father. **[This statement is TRUE: The proband inherited the 10.9-kb band (standard Southern blot with EcoR1/NruI double digestion) from her father and the 5.2-kb band from her mother. As a result, it is not surprising to observe the 10.9-kb band in the standard Southern analysis from the proband, when some of her father's X chromosome is fully methylated (inactivated), effectively abolishing the NruI site.]**

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

44. So-called "next-generation" sequencing innovations will have a significant impact on molecular genetic pathology. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:484-492; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. The low-scale, targeted gene/mutation analysis that currently dominates the clinical genetics field will ultimately be replaced by large-scale sequencing of entire disease gene pathways and networks. **[This statement is TRUE: This will be especially true for complex disorders.]**
b. The raw accuracy per nucleotide sequenced on the 454 Genome Sequencer FLX System (Roche Applied Science) is high in relation to Sanger sequencing. **[This statement is FALSE: The raw accuracy per nucleotide sequenced on the 454 FLX (99.5%), like all massively parallel sequencers, is low in relation to Sanger sequencing. However, extremely high-confidence base calls can be achieved by oversampling. The 454 FLX can sequence 100 Mb of DNA in 8 hours at an average read length of 250 bp.]**
c. The Illumina Genome Analyzer achieves parallelization by the *in situ* amplification of DNA fragments immobilized onto the flow cell of the instrument at a concentration that promotes a dense array of nonoverlapping fragment colonies. **[This statement is TRUE: The Illumina Genome Analyzer can sequence 600 Mb of DNA per day at an average read length of 36 bp.]**
d. The main advantage of the Applied Biosystems SOLiD System technology is that each base is interrogated twice, resulting in very accurate raw reads (greater than 99.9%) that require a lower amount of oversampling to reach a threshold value of confidence for base calling. **[This statement is TRUE: The Applied Biosystems SOLiD System generates approximately 500 Mb of sequence per day at an average read length of 35 bp.]**
e. The Helicos Heliscope is reportedly able to sequence up to 2,000 Mb per day, making it the highest throughput instrument currently available. **[This statement is TRUE: The process can produce reads up to 55 bp in length, although optimal coverage is obtained with shorter reads of 25-35 bp.]**

45. Given its limited multiplex capability, traditional PCR is an impractical method of genomic enrichment for next-generation sequencers. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:484-492; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Megaplex PCR, which primes with oligonucleotides immobilized onto a solid surface and uses universal primer sequences on the 5' ends of the immobilized primers to allow subsequent rounds of amplification in solution to amplify all products, currently captures 99% of targets per reaction. **[This statement is FALSE: Currently only 80% of targets are captured per reaction in megaplex PCR.]**
b. In the molecular inversion probe (MIP) protocol, the termini of the probes duplex with the complementary genome sequence, forming a circle with a 1-bp gap corresponding to the polymorphic base. **[This statement is TRUE: DNA ligase and mononucleotides are then added to each of four tubes containing the reaction mixture. For each probe, circle formation is accomplished in the tube containing the free nucleotide that is complementary to the genomic base that spans the gap. Circularized probes are then enriched by treatment with exonucleases that specifically digest linear DNA, followed by an amplification reaction.]**
c. Connector inversion probe (CIPer) is a modification of the MIP protocol. **[This statement is TRUE: In the CIPer strategy, all dNTPs are added to one tube and circles are formed after 3' extension to the 5' end of the probe approximately 100 bp downstream.]**

d. The main benefit of the gene-collector method is the high uniformity obtained among the targeted products, requiring less overall sequencing depth to obtain acceptable sequencing coverage for all targets. **[This statement is TRUE: In the gene-collector procedure, a high-complexity multiplex PCR is followed by enrichment using a gene-collector probe complementary to specific PCR primer ends, allowing for the circularization of targeted fragments.]**
e. In nucleic acid pull-down assays, targeted regions of the genome are selected by direct hybridization to oligonucleotide microarray probes. **[This statement is TRUE: Oligonucleotide microarray pull-downs appear to be amenable to large-scale capture of regions of interest.]**

46. Data management and storage and the ability to interpret the data present imposing obstacles to the clinical utility of next-generation sequencing. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:484-492; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. A laboratory running a high-throughput instrument 3 times per week could be expected to spend in excess of $100,000 per year in hardware alone to store, access, and back up the more than 100 terabytes of total data generated. **[This statement is TRUE.]**
b. Certain state and professional regulations specify that test results should be archived for at least five years. **[This statement is TRUE: Some recommendations call for archiving of genetic test results for 10 to 20 years.]**
c. Greater than 25,000 deleterious mutations of the *BRCA1* gene have been recorded in a database of *BRCA1* complete gene sequences derived from over 150,000 people. **[This statement is FALSE: At the Myriad Genetics laboratory, the *BRCA1* and *BRCA2* genes have been sequenced completely in over 150,000 people. Nearly 10,000 deleterious mutations and missense variants of negligible or uncertain clinical significance have been identified and recorded in a database.]**
d. At least for the foreseeable future, any next-generation sequencing program is likely to reveal more novel variants of uncertain significance than clear-cut mutations, making the test reporting immensely complicated. **[This statement is TRUE: The term "incidentalome" refers to the constellation of potentially spurious findings of novel variants unveiled in both affected patients and healthy individuals, which can potentially create uncertainty and anxiety with no obvious clinical benefit or intervention to be offered.]**
e. A missense change initially designated as a pathologic mutation may turn out, on further study, to be a benign polymorphism. **[This statement is TRUE: Given the nature of the genetic code, the physicochemical similarity of classes of amino acids, and negative evolutionary selection for changes that affect reproductive fitness, the number of benign variants is likely to far exceed the number of disease-causing mutations in most genes.]**

47. Inhibitors of the epidermal growth factor receptor (EGFR) have been found to be effective in the treatment of several human cancers. Based on the referenced Commentary and related article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:493-495 and J Mol Diagn 2008 10:520-526; the authors of the referenced articles did not disclose any potential conflicts of interest.]

a. Kinase inhibitors such as erlotinib and gefitinib have been most widely used in patients with lung adenocarcinoma. **[This statement is TRUE.]**
b. Anti-EGFR antibodies such as panitumumab and cetuximab are more widely used in colorectal cancer and cancers of the head and neck than in lung cancers. **[This statement is TRUE.]**
c. *EGFR* mutations are currently the most specific predictor of erlotinib or gefitinib response in patients with non-small cell lung cancer (NSCLC). **[This statement is TRUE: Approximately 80% of patients with an activating *EGFR* mutation have a response to erlotinib or gefitinib.]**
d. EGFR immunohistochemical expression in lung cancer shows a strong direct relationship to *EGFR* mutation status. **[This statement is FALSE: EGFR protein expression is the least specific marker for detecting patients likely to respond to anti-EGFR therapy, with the majority of lung adenocarcinoma patients expressing EGFR to some degree but only about 10% of patients responding to erlotinib or gefitinib. EGFR immunohistochemical expression shows little or no relationship to *EGFR* mutation status.]**
e. Some patients who respond to anti-EGFR therapies have negative results for all EGFR-related predictive biomarkers. **[This statement is TRUE: While it is possible that such discordant cases may reflect problems in the coverage or technical sensitivity of the *EGFR* mutation detection methods used, the data suggest a need for other markers that might refine or complement response prediction.]**

48. Mutations in the *KRAS* oncogene have been found in human cancers of the lung, colon, and pancreas. Based on the referenced Commentary and related article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:493-495 and J Mol Diagn 2008 10:520-526; the authors of the referenced articles did not disclose any potential conflicts of interest.]

a. KRAS is a downstream signaling molecule in the EGFR signaling pathway. **[This statement is TRUE: It is because of this downstream role of KRAS that initial studies examined it as a biomarker for resistance to EGFR-directed therapy.]**
b. The response rate for treatment with EGFR antibodies is significantly greater in patients with tumors with wild-type *KRAS* as compared with mutated *KRAS*. **[This statement is TRUE: Many oncologists now routinely request *KRAS* mutation testing to identify patients who should be offered non-anti-EGFR therapies.]**
c. In trials of single-agent panitumumab or cetuximab, the response rate of patients with *KRAS* mutation is 50%. **[This statement is FALSE: In trials of single-agent panitumumab or cetuximab, the response rate of patients with *KRAS* mutation is 0%.]**
d. Methods to routinely detect *KRAS* mutations in clinical samples include, among others, direct sequencing, PCR-restriction fragment length polymorphism, PCR-single strand conformation polymorphism, and mutant-allele-specific amplification. **[This statement is TRUE.]**
e. *KRAS* point mutations at codon 12 have been found in human colorectal, lung, and pancreatic cancers and may be useful biomarkers of resistance to EGFR-based therapeutics. **[This statement is TRUE: *KRAS* mutations at codon 12 have clinical relevance. Six nonsynonymous point mutations of codon 12 of the *KRAS* gene have been observed in human tumors: GAT, GCT, GTT, AGT, CGT, and TGT.]**

49. There is an increased demand for sensitive and rapid methods to detect the most common *KRAS* point mutations in routine clinical specimens. Based on the referenced Commentary and related article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:493-495 and J Mol Diagn 2008 10:520-526; the authors of the referenced articles did not disclose any potential conflicts of interest.]

a. The Smart Amplification Process version 2 (SMAP-2) is a single nucleotide polymorphism detection system that requires purification of DNA, involves many steps, and requires skilled technicians. **[This statement is FALSE: SMAP-2 can rapidly detect mutations from tissue and does not require DNA purification.]**
b. SMAP-2 utilizes a unique asymmetric design of the primers flanking the target sequence. **[This statement is TRUE: These primers are referred to as the folding primer and turn-back primer and are engaged in amplifying the target through a self-priming mechanism.]**
c. Peptide nucleic acid (PNA) is a synthetic oligonucleotide that cannot serve as a primer for polymerization because it is not recognized by the polymerase and cannot be a substrate for exonuclease activities of Taq polymerase. **[This statement is TRUE: The ribose-phosphate backbone of PNA is completely replaced by (2-aminoethyl)-glycine units linked by amide bonds.]**
d. The authors report a new method to detect all 6 possible clinically relevant *KRAS* mutations from only one primer set. **[This statement is TRUE.]**
e. SMAP-2 can be applied to paraffin-embedded archival tumor tissues as well as crude lysates of frozen tissue. **[This statement is TRUE.]**

50. The identification of breast carcinomas that are potentially responsive to targeted therapies based on *HER2* amplification relies on accurate and reproducible clinical laboratory assessment. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2008 10:527-536; the authors of the referenced article did not disclose any potential conflicts of interest.]

a. Currently, *HER2* status is determined by detection of the presence or absence of gene amplification assessed by *in situ* hybridization and/or by detection of the encoded protein by immunohistochemistry. **[This statement is TRUE: Fluorescence *in situ* hybridization (FISH) is the current reference standard technique for the assessment of *HER2* gene amplification.]**
b. According to the guidelines of the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP), *HER2* gene amplification status assessed by FISH is classified as positive with a *HER2*/chromosome 17 ratio higher than 1.8 on an average of 60 cells. **[This statement is FALSE: According to ASCO/CAP guidelines, *HER2* gene amplification status is classified as negative with an *HER2*/chromosome 17 ratio lower than 1.8, equivocal with a ratio between 1.8 and 2.2, and positive with a ratio higher than 2.2 on an average of 60 cells.]**
c. Silver *in situ* hybridization (SISH) is a high-sensitivity *in situ* hybridization technique based on enzymatic metallography and metallic silver deposition. **[This statement is TRUE.]**
d. The staining and interpretative reproducibility of the *HER2* SISH assay was compared with other *in situ* hybridization methods and immunohistochemistry for HER2 protein expression in five laboratories. **[This statement is TRUE: A total of 1,098 SISH-stained slides were evaluated. For comparison, all specimens were stained by 4B5 immunohistochemistry for HER2 protein expression.]**
e. The few discrepancies in observers' SISH scoring were attributable to chromosome 17 polysomy. **[This statement is TRUE.]**

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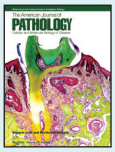
AJP CME Program in Pathogenesis



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