JMD CME Program in Molecular Diagnostics 2006

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

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CME Questions # 11-20
(See February 2006 Examination Sheet for Questions # 1-10)

11. "Break-apart" and "dual-fusion" probes refer to the two basic fluorescent probe designs used in fluorescent *in situ* hybridization (FISH). Based on the referenced Review article, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 141-151]

a. In contrast to single fusion probes, which are located close to the translocation breakpoint and have lower sensitivity, dual-fusion probes are designed to span the translocation breakpoint regions in the two different genes involved in a reciprocal translocation.
b. Dual-fusion probes are more informative for specific translocations because both loci involved in the translocation are labeled.
c. Break-apart probes may reveal unexpected variant translocations.
d. The genomic distance between break-apart probes may confound interpretation, increasing the chance of a false positive result.
e. Dual-fusion probes are superior to break-apart probes in every respect, and the latter should not be used for analysis of clinical specimens.

12. Artifactual truncation of nuclei during sectioning of tissue specimens is an important element to consider in applying FISH techniques to tissue sections. Based on the referenced Review article, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 141-151]

a. Although sections thicker than 6 microns may minimize the number of nuclei that are truncated during sectioning, they have some disadvantages, including the difficulty of interpreting signals in many different focal planes and distinguishing between individual nuclei.
b. Abnormal nuclei with loss of one or more fluorescent signals should never be interpreted
c. Truncation is more likely to be an issue with tumor cells displaying larger nuclei such as from a large cell lymphoma.
d. Truncation is a critical factor in determining appropriate "cut-off" values to be used for specific probes.
e. Truncation may confound interpretation of chromosomal deletions but is not problematic for the detection of amplifications.

13. Successful FISH demands attention to a number of key elements. Based on the referenced Review article, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 141-151]

a. Suboptimal duration and quality of fixation may prevent successful FISH.
b. Demasking procedures (the permeabilization of cells and nuclei by chemical or physical maneuvers to make nuclear sequences accessible to probes) may need to be modified for specific tissue specimens.
c. It is widely accepted that the diagnostic cut-off is calculated as the mean of false-positive findings in at least five healthy donors plus three times the standard deviation. Specific cut-off values need to be empirically determined for each probe, taking into account probe design, truncation artifact, chromatic condensation, and ploidy status.
d. Break-apart probes should ideally have a cut-off up to 15% when the distance between flanking signals is two or three times the estimated signal diameter.
e. Lymphomas may contain more than one chromosomal abnormality, and variant signal patterns should not be ignored.

14. The analysis of DNA methylation at the level of the individual nucleotide was revolutionized by the introduction of sodium bisulfite conversion of genomic DNA. Based on the referenced Commentary article, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 152-156]

a. Most approaches to interrogate for methylated DNA incorporate sodium bisulfite conversion to distinguish between cytosine and 5-methylcytosine.
b. The majority of 5-methylcytosine occurs in CpG dinucleotides, which are underrepresented in the mammalian genome but are enriched in specific regions of the genome, termed CpG islands.
c. Sodium bisulfite conversion of DNA is a gentle treatment that does not appreciably affect DNA degradation.
d. Sodium bisulfite conversion of DNA followed by polymerase chain reaction (PCR) and DNA sequencing is the gold standard for methylation analysis, but it is labor intensive, time consuming, relatively expensive, and not readily adaptable to high-throughput analysis of DNA samples.
e. The real-time PCR technique provides sensitive real-time detection of PCR products, eliminating the need for gel electrophoresis or other downstream analysis of PCR products and enabling analysis of minute quantities of DNA.

15. Identification and quantitation of methylation has become a significant tool in epigenetic research and clinical diagnostics. Based on the referenced article concerning the precision and performance characteristics of sodium bisulfite conversion and real-time polymerase chain reaction (PCR) for quantitative DNA methylation analysis, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 209-217]*

a. The variances of run-to-run percentage of methylated reference (PMR) values were larger than the coefficients of variation of bisulfite-to-bisulfite values, possibly reflecting the introduction of other sources of variation such as different standard curves.
b. To prepare methylated genomic DNA for their studies, the authors treated human peripheral blood leukocyte DNA with the CpG methylase M. *Sss*I.
c. Normalizing methylation measurements using a control reaction is required for cross-sample quantitative comparison of methylation.
d. Genes that showed significant variations in repeated amplifications, such as *ACTB*, were superior control genes to genes such as *COL2A1*.
e. In colon cancer samples, promoter methylation of *CDKN2A*, *MLH1* and *MGMT* was strongly associated with loss of respective protein expression.

16. Tissue fixation for traditional histopathologic evaluation often compromises subsequent molecular testing, prompting the development of novel formulations. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 157-169]

a. Reliable immunohistochemical staining of tissue sections requires the use of crosslinking fixatives such as neutral buffered formalin.
b. Non-crosslinking organic fixatives such as methacarn are suitable for preservation of RNA species.
c. DNA isolated from formalin-fixed tissue has been shown to exhibit high rates of non-reproducible alterations in sequencing studies, an artifact not realized with tissues fixed with the organic fixatives methacarn or RCL2.
d. High molecular weight DNA, as long as 800 base pairs, suitable for polymerase chain reactions (PCR) can be extracted from tissues fixed in non-crosslinking fixatives such as methacarn and RCL2.
e. Potential disadvantages of the use of methacarn are its poor stability and its potential toxicity.

17. Analysis of gene expression with DNA microarrays is plagued with lack of concordance among results obtained using different array platforms. Based on the referenced article that analyzed variations in amplification and labeling protocols on gene expression results using T7-based methods, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 183-192]

a. A single total RNA sample was used to focus on variations introduced by differences in amplification methods without interference from biological variation.
b. In all cases, biotin-labeled cRNA targets were prepared utilizing conjugated biotin; however, in one arm of the study, CTP was also biotinylated, introducing sequence-dependent bias.
c. The length of the *in vitro* transcription reaction had no effect on the size or yield of labeled products.
d. The data suggest that transcripts actually present in a sample are not always amplified successfully.
e. The length of time of the reverse transcription step may account for an under-representation of 5’ probes from larger genes, possibly because longer reverse transcription steps lead to depletion of dNTPs and early termination of reverse transcription reactions.

18. Alveolar rhabdomyosarcomas are characterized by recurrent chromosomal translocations. Based on the referenced article that describes a real-time polymerase chain reaction (PCR) method for analysis of formalin-fixed paraffin-embedded archival specimens, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 202-208]

a. The investigators used a convenience sample; however, subsequent multivariate analysis confirmed that the convenience sample was not representative of the entire cohort.
b. PAX7-FKHR tumors tended to occur in younger patients, were locally less invasive, and were associated with a better outcome than PAX3-FKHR tumors.
c. The investigators used a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) method in which RT and PCR steps were performed in one reaction system and the FKHR primer served as primer for both RT and PCR steps.
d. PAX3-FKHR and PAX7-FKHR fusion transcripts vary in size and composition, thereby complicating the design of a definitive real-time RT-PCR assay for these fusion products.
e. Successful RT-PCR results were obtained in 76% of cases from formalin-fixed, paraffin-embedded samples that were at least thirteen years old.

19. Congenital cytomegalovirus (CMV) is the most common infectious cause of sensorineural hearing loss in children. Based on the referenced article that describes a sensitive polymerase chain reaction (PCR)-based method for detecting CMV in dried blood spots routinely stored by state public health laboratories, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 240-245]

a. The real-time PCR assay that was used to amplify CMV from the dried blood spots was not quantitative.
b. The authors modified the manufacturer's method for purifying DNA from dried blood spots to increase the sample size and recovery of the lysate.
c. The extraction and amplification of human CMV DNA from perinatal cards was sensitive and linear over five-log concentrations of viral load.
d. The real-time PCR assay was sensitive enough to detect CMV DNA in some samples from patients with undetectable CMV by routine viral culture.
e. Most patients with congenital CMV infection do not have clinical signs or symptoms of CMV disease at birth.

20. Allogeneic bone marrow transplant patients can be monitored by *in vitro* amplification of polymorphic genetic markers. Based on the referenced Consultation in Molecular Diagnostics article, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 288-294]

a. A single informative polymorphic short tandem repeat (STR) locus (between donor and recipient) can be used for accurate assessment of engraftment status following bone marrow transplantation.
b. For a donor and/or pre-transplant recipient sample with two discernable STR alleles, the genotyping peaks are typically of similar area and amplitude.
c. The skewed allelic ratio in the pre-transplant and 54-day post-transplant samples for some STR loci was likely due to the patient's underlying hematopoietic disease.
d. The post-transplant DNA chimerism studies suggesting minimal or no engraftment were not consistent with the morphologic examination of the bone marrow aspirate and biopsy from the same time points.
e. This clinical case illustrates the need for comprehensive evaluation of pertinent clinical and laboratory data during engraftment analysis to identify potential sources for error in interpretation of STR analysis

*__Disclosures__: One of the authors of J Mol Diagn 2006 8: 209-217 is a shareholder and Scientific Advisory Board Member of Epigenomics, AG, which has a commercial interest in the development of DNA methylation markers for disease detection and diagnosis. The work described in this manuscript was not supported by Epigenomics, AG.

SEE EXAMINATION ANSWER SHEET – NEXT PAGE

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CME Questions # 11-20

Examination Answer Sheet #2, Questions #11-20					
Answer	**a**	**b**	**c**	**d**	**e**
Question #11	❍	❍	❍	❍	❍
Question #12	❍	❍	❍	❍	❍
Question #13	❍	❍	❍	❍	❍
Question #14	❍	❍	❍	❍	❍
Question #15	❍	❍	❍	❍	❍
Question #16	❍	❍	❍	❍	❍
Question #17	❍	❍	❍	❍	❍
Question #18	❍	❍	❍	❍	❍
Question #19	❍	❍	❍	❍	❍
Question #20	❍	❍	❍	❍	❍

Name	
Email Address	
CME ID# (For office use only)	

Instructions for Completing and Submitting the Examination:

1. You must be registered for the JMD CME Program prior to submission or you may register along with submission of your first Examination Answer Sheet of the year. *
2. Fill in the appropriate circle for each question to indicate your answer.
3. Enter your name and email address.
4. Mail or fax this completed Examination Answer Sheet (along with your payment and CME Registration Form if you have not already registered*) to the AMP/ASIP JMD CME office.
5. Keep a copy of your Examination Answer Sheet for your records to compare with correct answers.
6. Your score and correct answers will be emailed to you within 14 days.**

* Register online at **www.asip.org/register.html** or you may submit your CME 2006 Registration Form with payment prior to, or along with, your first Examination Answer Sheet of the year. You may download the JMD CME Registration Form at **www.asip.org/CME/jmdCME.htm** or **www.amp.org/CME/jmdCME.htm.**
** You may mail or fax your completed Examination Answer Sheet from each issue of JMD in order to receive correct answers within 14 days, **OR** you may collect your completed Examination Answer Sheets throughout the year, and mail or fax to the AMP/ASIP JMD CME office at the completion of the 2006 CME year.

Deadline for receipt of CME 2006 Registration Form, all Examination Answer Sheets, and CME Evaluation Form: February 1, 2007.

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