

See end of this section for full program information and registration details.

JMD CME Program in Molecular Diagnostics 2007

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

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<http://jmd.amjpathol.org>
www.asip.org/CME/jmdCME.htm

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

CME Questions # 1-10

1. The consistent use of uniform nomenclature in the management of genomic data is critical for accurate and concise communication of diagnostic testing and genetic risk assessment. Based on the referenced Special Article on standardization of mutation nomenclature, select the ONE statement that is NOT true regarding the components needed to describe a single nucleotide substitution based on a coding DNA sequence: [See J Mol Diagn 2007 9:1-6]

- The first component of the description is the Genbank accession number and version number of the reference sequence used.
- A colon ":" is used to separate the reference sequence from the information that follows, without intervening spaces.
- The inclusion of the prefix "c" to indicate that this is a coding DNA sequence is optional.
- The nucleotide number of the reference sequence must be included.
- The final components of the description are the identification of the wild-type nucleotide followed by the symbol ">" indicating a change, which is in turn followed by the identification of the mutant nucleotide.

2. Based on the referenced article on standardization of mutation nomenclature, select the ONE statement that is NOT true regarding the preference for identification of mutations at the level of DNA as opposed to the level of amino acids: [See J Mol Diagn 2007 9:1-6]

- Descriptions at the amino acid level are usually inferred with no experimental proof and are not unequivocal.
- DNA sequence changes may have unexpected effects, resulting in different mechanisms for impaired gene function.
- Different DNA sequence changes can result in an identical mutation
- Specific amino acid mutations should not be included in the descriptions under any circumstances.
- Several one-letter amino acid codes, including A, C, G, and T, may be confused with nucleotide code when a variant is rare or unfamiliar to health care providers.

3. The diagnosis of myeloproliferative disorders (MPDs) can be complex and expensive. Based on the referenced article that describes a quantitative real-time PCR assay for the detection of the *Janus kinase 2 (JAK2) V617F* mutation, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:42-46]

- The V617F mutation in the JH2 domain of the *JAK2* gene has been identified in a high proportion of patients suffering from polycythemia vera, essential thrombocythemia, secondary polycythemia, and idiopathic myelofibrosis.
- The *JAK2* mutation is an acquired mutation and may only be present in a small number of cells within a sample.
- Identification of the *JAK2* mutation establishes the presence of a clonal disorder and distinguishes between secondary polycythemia (secondary erythrocytosis) and polycythemia vera.
- The JH2 domain of *JAK2* may play a direct role in the negative regulation of *JAK2* signaling.
- Compared to the amplification refractory mutation system (ARMS) method, the real-time PCR assay was slightly less sensitive.

4. The vast majority of surgical biopsy and resection tissues are available only in the form of formalin-fixed, paraffin-embedded (FFPE) specimens. This has severely compromised attempts to correlate clinical, histologic, and outcome data with information from comprehensive gene expression profiles. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:70-79]*

- a. Fresh-frozen tissues are currently the preferred source of high quality RNA for cDNA microarray genomic expression studies.
- b. A limitation of fresh-frozen tissues is that they do not provide sufficient morphological detail for accurate clinical diagnosis.
- c. The ability of formalin to cross-link RNA and proteins has been the putative explanation for the historically limited success in extracting high-quality total RNA from FFPE tissues for use in reverse transcription reactions.
- d. Monomethylol groups (CH₂OH) can be added to bases in the presence of formalin, which may interfere with reverse transcription and amplification reactions. Formalin may be responsible for the addition of monomethylol groups to the RNA bases, limiting the use of FFPE as the RNA source.
- e. RNA isolated from FFPE specimens cannot be used for quantitative determination of mRNA levels through real-time PCR.

5. Establishment of a reliable method for using RNA from FFPE tissue would provide an opportunity to obtain novel gene expression data from the vast amounts of available archived tissue. Based on the referenced article concerning a custom-designed 22,000 oligonucleotide array to analyze the gene expression profile of colonic epithelial cells isolated by laser capture microdissection (LCM) from FFPE archived samples, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:70-79]*

- a. The RNA processing system was adapted to analyze very limited amounts of homogeneous cells (as little as 5 ng of total RNA) that were acquired by LCM.
- b. Neither prolonged storage of the FFPE specimens nor delays in formalin fixation of the surgical specimen were primary factors resulting in decay of RNA quality
- c. The use of linear amplification was important in generating a sufficient amount of RNA for microarray analysis from the small microdissected samples.
- d. The comparison of gene expression profiles between FFPE samples and matched frozen tissue samples obtained from the same patient at the same surgical intervention provided good control, and the immediate snap-frozen sample ensured an optimal source of material for microarray analysis.
- e. Both the concordance of expressed genes between paired samples and the high correlation coefficient of expressed genes between frozen and FFPE samples confirmed the similarity of gene expression profiles, although results varied widely in RNA quality for the individual samples.

6. Data analysis based on screening of multiple genes and their expression levels is a highly complex process. Based on the referenced article that describes a multiplex PCR reaction to identify gene expression profiles in SRBCT, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:80-88]*

- a. Artificial neural networks (ANN) using a 39-gene panel were capable of predicting the diagnostic classifications of four of the SRBCT, although both sensitivity and specificity were less than 80%.
- b. A gene minimization procedure using all the tumor samples to identify the optimal gene set for minimal classification errors resulted in a panel of 31 genes.
- c. The leave-one-out ANN analysis using 31 genes demonstrated that all samples were diagnosed accurately.
- d. Multidimensional scaling analysis (MDS) using the 31-gene panel showed that the samples clustered closely according to the 4 different cancer categories.
- e. Hierarchical sample clustering based on the 31-gene panel generally supported the ANN results with the exception of one rhabdomyosarcoma, which did not localize with other tumors of that category.

7. The small round blue cell tumors (SRBCT) of childhood are difficult to diagnose by routine histology and current diagnostic protocols. Based on the referenced article that describes a multiplex polymerase chain reaction (PCR) to identify gene expression profiles that can differentially diagnose four of the most aggressive SRBCT, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:80-88]*

- a. The SRBCT of childhood include neuroblastoma, Ewing's family of tumors (EWS), non-Hodgkin's lymphoma, and atypical lipomatous tumor.
- b. Cytogenetic testing can be used to identify tumor-specific translocations or to detect evidence of gene amplification.
- c. Interphase fluorescence in situ hybridization (FISH) can be used to identify tumor-specific translocations or evidence of gene amplification and is a more rapid and less expensive technique than conventional cytogenetics.
- d. Immunohistochemical studies are generally limited to examination of single protein markers.
- e. The reverse transcription-polymerase chain reaction (RT-PCR) is a widely used method for detection of tumor-specific translocations.

8. The diagnosis of α 1-antitrypsin deficiency (AAT) is often delayed long after appearance of symptoms, emphasizing the need for a rapid, relatively inexpensive, and reliable detection method. Based on the referenced article describing a method for genotyping the common AAT deficiency alleles, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9: 99-104]

- The most common clinical manifestation of AAT is early-onset panacinar emphysema and chronic obstructive pulmonary disease.
- Although the protease inhibitor locus that codes for AAT is highly polymorphic, two common mutations constitute most of the disease-causing decreases in serum levels of AAT.
- The common AAT deficiency alleles are highly prevalent in Asian populations.
- Liver diseases including hepatitis, cirrhosis, and hepatoma, and possibly vasculitis, represent the other clinical spectrum of AAT deficiency.
- The onset of AAT deficiency symptoms is accelerated in heavy smokers and mutation-carriers subjected to severe air pollution.

9. Currently, the diagnosis of pseudoxanthoma elasticum (PXE) depends on some combination of demonstrated calcification of dermal dystrophic elastic fibers by skin biopsy, presence of angioid streaks in the retina, and positive family history. Based on the referenced article, which describes an approach for detection of the common mutations in the *ABCC6* gene associated with the diagnosis of PXE, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:105-112]

- The *ABCC6* gene consists of 31 exons on human chromosome 16p13.1 and encodes a protein belonging to the ATP-binding cassette membrane transporter family.
- PXE is a hereditary disorder that affects the skin and eyes, as well as occasionally the gastrointestinal and cardiovascular systems.
- The methods in the strategy described are capable of detecting mutations of exons 22 to 29 in the *ABCC6* gene, where the common mutations in PXE are localized.
- The methods described involve relatively small financial investment in equipment and instrumentation.
- The test can be performed directly with PCR products without further DNA purification.

10. Hereditary amyloidosis can be caused by mutations in one of several genes. Based on the referenced Consultations in Molecular Diagnostics article describing a homozygous transthyretin (TTR) mutation, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:127-131]

- Amyloidosis refers to a group of disorders that share the deposition in one or more organs of abnormal fibrillar proteins that are characterized by a beta-pleated sheet conformation.
- TTR is a serum protein produced in the liver that transports thyroxine and retinol-binding protein.
- Mutations in the TTR gene can destabilize the TTR molecule and are responsible for the most frequent form of hereditary autosomal-dominant amyloidosis.
- A common mutation in the African-American population is a point mutation at codon 122 of the TTR gene that causes a valine to isoleucine substitution at amino acid 122, resulting in amyloid deposition in the liver.
- Homozygosity for the valine to isoleucine substitution at amino acid 122 of TTR may be associated with earlier onset of cardiac disease.

***Disclosures:**

J Mol Diagn 2007 9:70-79: Some of the authors are employees of Molecular Devices, which recently purchased Arcturus Bioscience.

J Mol Diagn 2007 9:80-88: Some of the authors are affiliated with Althea Technologies.

J Mol Diagn 2007 9:105-112: Some of the authors are associated with PXE International, Inc. and Transgenomic, Inc., which would sponsor and distribute, respectively, a test kit (pending FDA approval) based upon the research described.

SEE EXAMINATION ANSWER SHEET – NEXT PAGE

**REGISTRATION INFORMATION FOLLOWS FOR
THE 2007 JMD CME PROGRAM IN MOLECULAR DIAGNOSTICS**

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CME Questions # 1-10

Examination Answer Sheet #1, Questions #1-10					
Answer	a	b	c	d	e
Question #1	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #2	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #3	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #4	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #5	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #6	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #7	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #8	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #9	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #10	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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 1 month.**

* Register online at www.asip.org/register.html or you may submit your CME 2007 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 1 month, **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 2007 CME year.

Deadline for receipt of CME 2007 Registration Form, all Examination Answer Sheets, and CME Evaluation Form: February 1, 2008.

Complete Journal CME 2007 Information, including the CME Conflict of Interest Disclosure Policy, is on the AMP and ASIP websites at: www.amp.org/CME/jmdCME.htm and www.asip.org/CME/jmdCME.htm

Direct all Inquiries to:

Salomé Creighton
 ASIP/AMP CME Administrative Assistant
 9650 Rockville Pike
 Bethesda, MD 20814-3993 (USA)
 Tel: 301-634-7942; Fax: 301-634-7990
 Email: ajpcme@asip.org