

JMD CME Program in Molecular Diagnostics 2006

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CME Questions # 31-40

(See February-July 2006 Examination Sheets for Questions #1-30)

31. The widespread occurrence of a somatic, acquired point mutation in a highly conserved residue of the auto-inhibitory domain of the Janus kinase 2 (JAK2) tyrosine kinase provides new insight into the pathobiology of myeloproliferative disorders (MPDs). Based on the referenced Review article that describes tests to detect the genetic mutation underlying the V617F point mutation of JAK2, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 397-411]

- a. JAK2 plays an important role in normal hematopoietic growth factor signaling.
- b. The JAK2 V617F mutation causes constitutive activation of the kinase.
- c. Patients with clonal MPD may exhibit JAK2 V617F mutations.
- d. In addition to somatic, acquired V617F mutations, germline JAK2 mutations have been detected in many patients with hematopoietic malignancies.
- e. In most cases, the JAK2 V617F mutation confirms a diagnosis of polycythemia vera.

32. JAK2 V617F has shown a role in the diagnostic algorithms for specific patient scenarios. Based on the referenced Review article that describes useful diagnostic techniques for detection of V617F, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 397-411]

- a. Sequencing using the fluorescent dye-terminator variant of the Sanger sequencing methods (Big Dye) is preferred for its sensitivity.
- b. Small amounts of mutant DNA in a wild-type background are most easily detected using allele-specific polymerase chain reaction (PCR) or the amplification refractory mutation system (ARMS).
- c. Real-time PCR using DNA melting curve analysis is a useful screening test to initially evaluate patients with erythrocytosis.
- d. Restriction fragment length polymorphisms (RFLPs) can be used to elaborate JAK2 V617F mutation.
- e. Pyrosequencing is a method of rapid genotyping that depends on the liberation of pyrophosphate whenever a dNTP is incorporated into a growing DNA chain during template-driven DNA polymerization; it has the advantage of allowing estimation of allelic ratio in hematopoietic chimerism or in mixed clonality/heterogeneous tissue samples, which is characteristic of MPDs.

33. Antigen receptors are central components of the adaptive immune response. Based on the referenced Commentary article, select the ONE statement regarding the mechanisms underlying the generation of a diverse immune response that is NOT true: [See J Mol Diagn 2006 8: 426-429]

- a. There are a large number of genes encoding immunoglobulins and T-cell receptors for B and T cells, respectively.
- b. Gene rearrangement is a major mechanism for the generation of a diverse immune response.
- c. Terminal deoxynucleotidyl transferase (TdT) deletes N-nucleotides in tandem with the rearrangement process.
- d. TdT inserts N-nucleotides in tandem with the rearrangement process.
- e. Somatic hypermutation is restricted to the germinal center phase of B-cell development.

34. Analysis of antigen receptor genes has been central to the assessment of the clonality of lymphoproliferative disorders. Based on the referenced Commentary article, select the ONE statement

regarding the components of the gene arrangement process that is NOT true: [See J Mol Diagn 2006 8: 426-429]

- a. Segments in all antigen receptor genes that are shuffled during rearrangement include variable (V), diversity (D) and joining (J) regions.
- b. Recombination signal sequences flank the V, D, and J regions.
- c. The process of rearrangement is initiated by a specific complex that includes proteins of the recombination activating genes.
- d. Recombination signal sequences are specific sequences that are 7 or 9 bases long.
- e. Rearrangements can occur beyond very early lymphoid development.

35. Based on the referenced Commentary article, select the ONE statement regarding the clonality of lymphoproliferative disorders that is NOT true: [See J Mol Diagn 2006 8: 426-429]

- a. A monoclonal population of lymphocytes is derived from a single tumor cell and will spawn a homogeneous population of cells that evince an identical gene rearrangement.
- b. Techniques that can be used to distinguish monoclonal populations from each other include Southern blotting and polymerase chain reaction (PCR).
- c. Documentation of an antigen receptor gene rearrangement in B- and T-cell neoplasms provides a molecular fingerprint of a neoplasm that can be relied upon for definitive minimal residual disease assessment.
- d. Lineage infidelity (or lineage promiscuity) refers to cross-lineage rearrangements in the same cell of a B- or T-cell neoplasm and is more prevalent in immature or precursor neoplasms than in more differentiated or peripheral neoplasms.
- e. It is recommended for minimal residual disease analysis that two or more different antibody receptor loci should be tracked to increase the likelihood that at least one stable, retained rearrangement can be detected throughout the course of the disease.

36. Peripheral T-cell lymphoma (PTCL) is an uncommon malignancy with a difficult diagnosis. Based on the referenced article concerning the detection of B- and T-cell clonality in PTCL, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 466-475]

- a. Factors that make the diagnosis of PTCL difficult include non-specific histologic findings that can overlap with reactive conditions, the lack of good immunophenotypic markers to establish clonality, and the presence of B-cell clones in some cases.
- b. A high overall frequency of B-cell clones was found in both PTCL and angioimmunoblastic T-cell lymphoma (AITL).
- c. A larger than expected number of rearrangements in V γ 10 was found.
- d. Cases of PTCL and AITL demonstrated a high frequency of B-cell clones that correlated with EBV.
- e. One factor contributing to the high frequency of detected B-cell clones may be the inherent sensitivity of the PCR, which used primers targeting all three framework region primer sets.

37. ViroSeq mutation detection is a population sequencing-based HIV-1 genotyping method for antiretroviral drug resistance mutations present in the patient sample. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 430-432]*

- a. Women who receive a single dose of the antiretroviral drug nevirapine commonly demonstrate selection for variants with the K103N nevirapine resistance mutation.
- b. The LigAMP assay is based upon mutation-specific ligation of two adjacent oligonucleotides hybridized to a DNA template.
- c. LigAMP is a highly sensitive point mutation assay capable of detecting and quantifying HIV-1 minority variants.
- d. Paired plasmids with and without the K103N mutation were used as reference reagents for each subtype of HIV-1.
- e. ViroSeq consistently detected the K103N mutation in plasma samples with HIV-1 subtypes A, C, and D at levels over 20% of the viral population but did not detect the K103N mutation at levels less than 10% of the viral population.

38. The genetic heterogeneity of deafness has proved a challenge for genetic testing. Based on the referenced article concerning a microarray-based approach, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 483-489]

- a. Approximately 0.1% of children are born with a prelingual hearing loss that can have significant impact on the infant's speech, language and communication skills.
- b. Approximately 10% of prelingual deafness cases have a genetic basis.
- c. The "Hearing Loss Biochip" used in the current study allowed the parallel analysis of 15 common mutations or polymorphisms in four genes.
- d. The current version of the "Hearing Loss Biochip" was not designed to detect 12 mutations that were detected by sequencing of the connexin 26 gene (*GJB2*) in the 250 patients in the study set.
- e. A possible complication for genetic counseling of affected families is that deaf people with only one detectable *GJB2* mutation may be a carrier of a recessive mutation, with hearing loss caused by environmental or other genetic factors.

39. Although fine-needle aspiration of suspect thyroid lesions is currently the standard procedure to triage patients for surgical resection, significant limitations remain. Based on the referenced article describing microarray analysis of thyroid nodules, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 490-498]

- a. PAX-PPAR γ translocations and BRAF mutations are both commonly found in papillary thyroid carcinomas.
- b. Immunohistochemical analysis of fine needle aspirate cytology specimens suffers from the lack of established diagnostic markers.
- c. Microarray analysis of the fine needle aspirate samples clustered the test set into three groups: a malignant group, a benign group, and an indeterminate group. The five samples in the latter group were deemed suspicious on pre-operative fine needle aspiration, and final histological review showed cytologic heterogeneity in most of the samples.
- d. The classification of nodules as benign or malignant by microarray analysis of fine needle aspirates was 100% concordant to the final histology diagnosis of the surgical specimen.
- e. Although thyroid nodules are clinically detectable in less than 8% of the population, they are detected in approximately 50% of autopsy specimens.

40. Multicolor chromosome banding (mBAND) is a recently developed technique that allows the delineation of chromosomal regions. Based on the referenced Consultations in Molecular Diagnostics article that describes complex intrachromosomal rearrangements of chromosome 18 in a child with dysmorphic features, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 521-525]

- a. Molecular cytogenetic techniques including fluorescence *in situ* hybridization and spectral karyotyping have been used as complementary tools for analysis of complex chromosome rearrangements involving more than two chromosomes or more than two breaks in one chromosome, and microarray comparative genomic hybridization has been used to define critical regions for deletion or duplication.
- b. Isolated paint probes used in spectral karyotyping cannot be used to identify intrachromosomal rearrangements such as reciprocal translocations, inversions, or insertions.
- c. The mBAND probe is a DNA probe that contains a mix of region-specific partial chromosome paint probes generated by microdissection of a particular chromosome and labeled with three to five different fluorophores.
- d. In the case described, cytogenetic and FISH analysis indicated a mosaic karyotype in which one set of cells had a ring chromosome composed of chromosome 18 material and the other cell line had a partial trisomy for 18p and a partial monosomy for 18q. mBAND results were consistent with these analyses for the ring chromosome but showed a larger region of 18q deletion and a larger region of 18p duplication in the cell line with the derived chromosome 18.
- e. The resolution of mBAND is higher than that of conventional chromosome banding.

***Disclosures:**

J Mol Diagn 2006 8:430-432: One of the authors is an employee of, and another author received research support from, Celera Diagnostics, the manufacturer of the ViroSeq system. Three of the authors are co-inventors of the LigAmp assay and their institution has filed a patent application with the United States Patent and Trademark Office. The inventors may receive royalty payments if the patent is awarded and licensed.

SEE EXAMINATION ANSWER SHEET – NEXT PAGE

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CME Questions # 31-40

Examination Answer Sheet #4, Questions #31-40					
Answer	a	b	c	d	e
Question #31	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #32	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #33	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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Question #37	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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Question #39	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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Name					
Email Address					
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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.**

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