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American Society for Investigative Pathology

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www.asip.org/CME/jmdCME.htm

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CME Questions # 31-40

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

31. The use of real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) for measuring *BCR-ABL1* transcripts has become standard methodology for the diagnosis and monitoring of minimal residual disease in patients with chronic myeloid leukemia (CML). Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:421-430]*

- CML is a stem cell disease that is associated with a reciprocal chromosomal translocation t(9;22)(q34;q11) called the Philadelphia chromosome.
- The *BCR-ABL1* fusion gene is present in 99% of adult patients with acute lymphoblastic leukemia (ALL).
- Fusion *BCR-ABL1* transcripts encode oncoproteins which enhance tyrosine kinase activity and play a critical role in the pathogenesis of CML.
- Data from the IRIS and TIDEL trials indicate that molecular response, as measured by real-time qRT-PCR, can predict overall and progression-free survival after imatinib treatment.
- In addition to real-time qRT-PCR, conventional cytogenetic analysis, fluorescent *in situ* hybridization (FISH), and reverse transcription PCR (RT-PCR) can be used to detect the *BCR-ABL1* rearrangement.

32. Comparison of results of quantitative testing for *BCR-ABL1* from laboratories using different platforms, internal controls, reagents and calculation methods showed that there can be considerable variability of results from laboratory to laboratory. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:421-430]*

- In the North American survey of laboratories using real-time qRT-PCR to monitor CML patients, real-time qRT-PCR was used to measure RNA transcript levels in unknown diluents of the K562 cell line.
- In the North American sample exchange, samples were shipped to each laboratory on dry ice and laboratories were asked to store samples at -80°C until use.
- Each laboratory that participated in the North American sample exchange and survey used its own RNA extraction method.
- Laboratories which used *ABL1* as the internal control experienced significantly different results from other laboratories.
- It is recommended that each laboratory optimize its protocol for minimal residual disease monitoring.

33. Ewing family tumors (EFTs) are molecularly characterized by expression of chimeric transcripts generated by specific chromosomal translocations, most commonly involving fusion of the *EWS* gene to a member of the ETS family of transcription factors. Based on the referenced articles and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:437-440, J Mol Diagn 2007 9:459-463, and J Mol Diagn 2007 9:498-509]

- The most frequent translocation involving EFTs involves the *EWS* gene on chromosome 22 and the *ERG* gene on chromosome 21.
- The ETS family of transcription factors includes FLI1, ERG, ETV1, E1AF, and FEV.
- The ETS family of transcription factors is defined by the ETS domain, which interacts with DNA at sequences containing a common sequence motif.
- EWS* is a ubiquitously expressed protein containing an RNA-binding domain in its C-terminal portion.
- The *EWS* promoter is strongly and broadly activated, leading to the relatively unrestricted high level expression of the resulting fusion genes, while expression of native FLI1 is tightly regulated and lineage restricted.

34. Classic Ewing sarcoma is a primitive bone sarcoma of children and adolescents. Based on the referenced articles and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:437-440, J Mol Diagn 2007 9:459-463, and J Mol Diagn 2007 9:498-509]

- a. Ewing sarcoma is the second most common bone malignancy of childhood.
- b. Ewing sarcoma is more prevalent in males than in females.
- c. Ewing sarcoma does not occur in the elderly.
- d. Malignant cells that are characteristic of the Ewing family of tumors display intense cytoplasmic membrane-associated immunoreactivity with antibodies to CD99.
- e. EFTs include classic Ewing sarcoma of bone, extra-osseous Ewing sarcoma, peripheral primitive neuroectodermal tumor, and Askin tumor.

35. The existence of a growing number of rare EWS variant translocations complicates the molecular diagnosis of this group of undifferentiated small round cell sarcomas. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:437-440 and J Mol Diagn 2007 9:459-463]

- a. A novel t(2;16) translocation producing an in-frame fusion of *FUS* and *FEV* was identified in a 33-year-old male with a pathological fracture of the distal clavicle.
- b. Variant translocations involving EWS homologs involve so-called “promiscuous” molecular partnerships that may lead to false-negative results during diagnostic evaluation if the appropriate probes are not used.
- c. It is possible that the frequency of *FUS* rearrangement is under-reported because its possible involvement in EFTs is not routinely considered.
- d. The chromosome breakpoints involved in *EWS* to *FLI1* fusions are restricted to one intron in the *EWS* gene and three introns in the *FLI1* gene, giving rise to a limited number of possible EWS-FLI1 fusion products.
- e. The reported novel case of a *FUS-FEV* fusion incorporates a greater number of *FUS* exons than previously reported for *FUS-ETS* gene fusions.

36. Most well-known EWS fusions that have not involved the ETS family of transcription factors have been described in tumors that are pathologically distinct from EFTs. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:437-440 and J Mol Diagn 2007 9:498-509]

- a. Alveolar rhabdomyosarcomas contain fusions of FKHR to members of the PAX gene family.
- b. Extraskelatal myxoid chondrosarcomas contain fusions of the TEC transcription factor to a member of the TET family of RNA-binding proteins.
- c. Clear cell sarcomas contain fusions of *EWS* to the CREB transcription factor family.
- d. Tumors with related variant fusions tend to be more similar to each other than to tumors with completely different fusions.
- e. The authors report a novel case of *EWS-SP3* fusion, which involved the carboxy-terminal portion of EWS fused to the SP3 zinc-finger DNA-binding domain.

37. The multitude of EFT-associated fusions impacts on the choice of testing methodology. Based on the referenced articles and related Commentary, select the ONE statement related to molecular diagnosis of sarcomas that is NOT true: [See J Mol Diagn 2007 9:437-440, J Mol Diagn 2007 9:459-463, and J Mol Diagn 2007 9:498-509]

- a. Standard cytogenetic analysis has a low failure rate in sarcomas.
- b. RT-PCR procedures can be performed on fresh, frozen, or formalin-fixed, paraffin-embedded (FFPE) tissues and are amenable to being performed in batches.
- c. For fresh or frozen tissues, the isolated RNA is usually sufficiently intact and RT-PCR assays can be utilized that will detect the full range of fusion transcript sizes.
- d. For fresh or frozen material, three RT-PCR reactions utilizing specific sets of primers may suffice to detect the five fusions that involve *EWS* and ETS transcription factor family genes.
- e. In FFPE specimens, RNA is likely to be less intact due to hydrolytic events and crosslinking.

38. Fluorescent *in situ* hybridization (FISH) assays are part of the molecular testing paradigm for sarcomas. Based on the referenced articles and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:437-440, J Mol Diagn 2007 9:459-463, and J Mol Diagn 2007 9:498-509]

- a. FISH can be applied to fresh, frozen, or FFPE specimens.
- b. The split probe FISH *EWS* assay utilizes differentially labeled break-apart probes flanking the two sides of the *EWS* breakpoint region to determine when a rearrangement event involving *EWS* has occurred.
- c. The fusion FISH *EWS* assay, which uses differentially labeled probes from two chromosomal loci, has fewer false positives than the split probe assay.
- d. A potential problem with the FISH *EWS* split probe assay is that it cannot provide definitive proof of an EFT-associated gene fusion.
- e. RT-PCR assays can be a useful adjunct to FISH assays.

39. Post-plebotomy changes in the transcription profile caused by degradation and gene induction can lead to inconsistent nucleic acid-based results. Based on the referenced Technical Advance article concerning a procedure for isolation of RNAs and DNA, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:452-458]*

- a. The PAXgene Blood RNA stabilization and isolation procedure is dedicated to the isolation of mRNA, rRNA, and tRNAs.
- b. Microarray expression analysis of whole blood RNA presents a great challenge since reticulocyte transcripts may contribute up to 70% of all transcripts, of which most are globin transcripts.
- c. The Tri-X isolation method allows the collection of genomic DNA and RNA species of various sizes.
- d. No significant deterioration of total RNA was observed during 2.5 years of storage in PAXgene Blood RNA Tubes.
- e. For the microarray labeling reaction, an ethanol precipitation step was necessary to increase the concentration of eluted small RNAs.

40. Aberrant DNA methylation patterns have been identified in a variety of human diseases, including cancer. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:510-520]

- a. Pyrosequencing technology is based on the luminometric detection of pyrophosphate that is released upon nucleotide incorporation.
- b. Pyrosequencing technology has been used as a diagnostic test for aberrant methylation in imprinting disorders such as Prader-Willi and Angelman syndrome.
- c. Although serial pyrosequencing can reduce cost, labor and analysis time as well as saving precious DNA samples for the analysis of a specific region amplified in a single PCR, it still requires prior knowledge of the presence of epimutations in the target.
- d. Global hypermethylation is a hallmark of cancer.
- e. Genes involved in DNA repair, detoxification, cell cycle regulation, and apoptosis are often inappropriately inactivated in cancer cells due to hypermethylation of CpG islands.

***Disclosures:**

J Mol Diagn 2007 9:421-430: Two of the authors are consultants for Novartis, Canada.

J Mol Diagn 2007 9:452-458: Two of the authors are affiliated with Qiagen Inc. TRI-X is intended for research use only. No claim or representation is intended for its use to provide information for the diagnosis, prevention, or treatment of a disease.

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"/>
Question #34	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #35	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #36	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #37	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #38	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #39	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #40	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Name					
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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.**

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** 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.

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