

JMD CME Program in Molecular Diagnostics 2006

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

The Journal of Molecular Diagnostics, Volume 8, No. 3 (July 2006)

<http://jmd.amjpathol.org>

www.asip.org/CME/jmdCME.htm

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CME Questions # 21-30

(See February and May 2006 Examination Sheets for Questions #1-20)

21. The introduction of molecular diagnostic tests for invasive fungal infections may lead to earlier and more accurate and specific diagnosis in the immunocompromised population. Based on the referenced Commentary article, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 297-298, a Commentary on J Mol Diagn 8: 376-384]*

- The approach to develop consensus on polymerase chain reaction (PCR)-based tests for fungal disease bears little resemblance to earlier efforts to optimize molecular viral diagnostics.
- The aim of the UK-Irish Fungal PCR Consensus Group is to reach consensus regarding PCR-based tests for the diagnosis of invasive fungal infections.
- Numerous studies have demonstrated limitations of qualitative fungal PCR assays, including lack of specificity and the inability to predict invasive fungal disease.
- Lack of specificity in molecular assays for invasive fungal disease are most likely secondary to transient fungal colonization, mucosal disease such as thrush, or fungal spores that may contaminate a specimen.
- The development of quantitative approaches for detection of invasive fungal infections such as *Candida* and *Aspergillus* may permit the ability to distinguish results with high predictive value for invasive infections from results of low predictive value due to transient colonization and sample contamination.

22. Limitations of classical diagnostic methods for invasive fungal infections have led to the development of molecular techniques. Based on the referenced article concerning the detection of systemic fungal infections, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 376-384]*

- Improvements in medical therapies have led to a significant increase in the population of immunocompromised patients who are susceptible to invasive fungal infections.
- While the diagnosis of most invasive fungal infections is difficult, classical culture techniques provide excellent sensitivity for invasive aspergillosis.
- Histopathology can provide definitive evidence of invasive fungal infection but cannot always identify the causative organism.
- The current study focuses on amplification methods and does not evaluate specific methods for nucleic acid extraction from fungi.
- The multi-center study of PCR methods found that all evaluated methods for detection of *Candida* were comparable with a satisfactory level of detection of 10 cfu per mL blood.

23. Based on the referenced article that describes amplification methods for *Aspergillus fumigatus*, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 376-384]*

- To minimize variables in evaluating the various PCR methods, DNA extracts and the oligonucleotides utilized as primers were distributed to all participating centers in the multicenter study.
- The performances of the two optimal *Aspergillus* assays varied with sample type and platform.
- One set of reactions utilized a forward primer that was discovered to have homology with the *Homo sapiens* 18S rRNA gene, with consequent amplification of some human DNA.
- An advantage of a pan-fungal primer is that it could be used to detect emerging infections caused by other fungal pathogens (eg, *Fusarium* and *Scedosporium* species) without affecting sensitivity for *Aspergillus* and *Candida*.
- It may be possible to develop a strategy covering a range of invasive fungal infections by combining pan-fungal and species-specific methods.

24. Chronic myeloproliferative diseases (CMPD) are a group of related hematopoietic stem cell proliferation disorders. Based on the referenced Technical Advances article concerning the detection of an activating mutation in paraffin-embedded trephine bone marrow biopsies of some patients with CMPD, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 299-304]

- a. CMPD include chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), chronic idiopathic myelofibrosis (CIMF) and rarer entities such as idiopathic hypereosinophilic syndrome/chronic eosinophilic leukemia and chronic neutrophilic leukemia.
- b. Bone marrow changes associated with CMPD include hyperplasia of one or more hematopoietic cell lines, dysplastic features predominantly of the megakaryocytic lineage, fibrosis, and changes of bone marrow microvasculature.
- c. The recent identification of the recurrent V617F mutation in exon 12 of janus kinase 2 (JAK2) in a high proportion of patients with CML, PV, ET, and CIMF provides an important diagnostic marker.
- d. One of the assays used to detect the V617F mutation employed nested PCR of paraffin-embedded trephine bone marrow biopsies to amplify the region containing the mutation and subsequent restriction enzyme digestion with *BsaXI*.
- e. The V617F mutation was detected in 96% of PV, 74% of ET, 62% of CIMF, and 75% of unclassified CMPD cases tested.

25. Microsatellite instability (MSI) analysis of colorectal cancers is clinically useful to identify patients with hereditary non-polyposis colorectal cancer (HNPCC) caused by germline mutations of mismatch repair genes and may also predict cancer response and resistance to certain chemotherapies. Based on the referenced article concerning a comparison of two assays for MSI, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 305-311]*

- a. MSI is caused by DNA mismatch repair deficiency resulting in failure to repair the errors that normally occur during replication of repetitive DNA sequences.
- b. MSI only occurs in the presence of germline defects in mismatch repair genes, including *hMLH1*, *hMSH2*, *hMSH6*, and *hPMS2*.
- c. The Bethesda panel is a reference panel of five microsatellite markers for the detection of MSI that includes two mononucleotide loci and three dinucleotide loci.
- d. The MSI Analysis System utilizes five nearly monomorphic mononucleotide microsatellite loci for MSI determination and two polymorphic pentanucleotide markers for sample identification.
- e. The overall concordance between the Bethesda panel assay and the MSI Analysis System was 85%.

26. Studies of somatic mitochondrial DNA mutations have become an important aspect of cancer research since these mutations might have functional significance and/or serve as biomarkers for tumor detection. Based on the referenced article describing the analysis of prostate cancers, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 312-319]*

- a. Complete mitochondrial genome sequencing was performed on samples from malignant glands, adjacent benign glands, and distant benign glands in prostatectomy samples, in addition to control samples.
- b. Mutations were restricted to histologically malignant glands.
- c. Somatic mtDNA mutations were found frequently in prostate cancer samples.
- d. Most somatic mutations found in histologically benign samples were confined to the non-coding region.
- e. There appeared to be a progressive pattern of mtDNA mutations in malignant disease such that upon development of an adenocarcinoma, the mutation load increased in the coding region.

27. Metastatic disease is present without an identifiable primary tumor site in 3 to 5% of all cancers. Based on the referenced article that describes a quantitative reverse transcriptase (RT)-PCR assay to identify metastatic carcinoma tissue of origin, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 320-329]*

- a. Cancers of the lung, pancreas, and colon are among the most prevalent carcinomas of unknown primary origin.
- b. A set of ten tissue-specific markers for lung, colon, pancreatic, breast, prostate and ovarian carcinomas were selected from twenty-three putative candidates after validation by RT-PCR on formalin-fixed, paraffin-embedded metastatic carcinoma specimens.
- c. A predictive algorithm was built after quantitative RT-PCR assay optimization on a "training set" of known metastatic carcinomas.
- d. Coagulation factor V was identified as a novel pancreatic cancer-specific marker.
- e. Shortening the proteinase K incubation time from 16 hours to 3 hours had no effect on yield or on the quality of isolated RNA.

28. Monitoring multiple myeloma patients for relapse requires sensitive methods to measure minimal residual disease. Based on the referenced article that describes a real-time quantitative PCR test for the *IgH* gene, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 364-370]

- After stem cell transplantation, less than half of multiple myeloma patients achieve complete remission with negative immunofixation.
- Hypermutation in the VDJH rearrangement poses a major problem in multiple myeloma real-time PCR quantification.
- The so-called "Self-quenched PCR" refers to a technique that uses a self-quenched primer in which only two oligonucleotides are used in PCR, avoiding problems derived from the use of the third oligonucleotide (the probe).
- In all patients, the Self-quenched PCR technique exhibited significantly increased sensitivity, reproducibility, and quantification curve efficiency compared to the allele-specific *IgH* real-time quantitative PCR technique using a TaqMan probe and a JH consensus primer.
- In some patients, the efficiency and sensitivity of PCR were inadequate no matter which technique was used, due to the presence of somatic mutations in the target for either the probe, the JH intronic primer, or the JH self-quenched primer.

29. In the Caucasian population, cystic fibrosis (CF) is the most common genetic disease affecting life span. Based on the referenced article that assesses two commercially available analyte-specific reagents capable of testing for CF mutations, select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 371-375]*

- The American College of Medical Genetics (ACMG) and the American College of Obstetrics and Gynecology (ACOG) have published guidelines for population-based screening for CF. Current ACMG guidelines recommend CF carrier detection screening using a panel of 23 mutations and 4 polymorphisms in the cystic fibrosis transmembrane regulator gene and ACOG guidelines recommend that screening be offered to all Caucasian couples and that couples of other races be informed of the availability of CF carrier detection.
- Screening Caucasian individuals using the ACMG-recommended panel is expected to identify all Jewish CF carriers.
- There were no discrepant results in over 1,000 consecutive analyses between the two commercially available platforms.
- No additional mutations to the 23 that constitute the ACMG-recommended panel were detected in a general population of 1,029 patients.
- The Luminex bead system offers a sufficiently large number of assays to accommodate the ACMG-recommended CF screening panel as well as testing panels for other diseases prevalent among individuals of Ashkenzai Jewish descent.

30. Application of an appropriate internal normalization control is essential for accurate determination of clinically relevant RNA levels due to the variation of quality and quantity of RNA derived from clinical samples. Based on the referenced article that describes a control gene for molecular monitoring of chronic myelogenous leukemia (CML), select the ONE statement that is NOT true: [See J Mol Diagn 2006 8: 385-389]

- The method of choice for monitoring of CML is real-time quantitative PCR of *BCR-ABL* transcripts.
- Criteria to be applied to the evaluation of internal normalization control genes include absence of pseudogenes, RNA expression levels in various tissues and cell types, and RNA stability.
- ABL* is involved in various signaling pathways initiated by growth factors, DNA damage, and oxidative stress and in some cases levels of *ABL* mRNA have increased after treatment with Gleevec.
- Using novel criteria that are emphasized in residual disease testing, the authors found that *glucose6-phosphate dehydrogenase (G6PD)* is the most suitable control gene for *BCR-ABL* quantification.
- The authors selected *β-glucuronidase (GUSB)* as the control gene of choice for *BCR-ABL* quantification.

***Disclosures:**

J Mol Diagn 2006 8:305-311: One of the authors was the principal investigator in developing the assay and is affiliated with Promega Corporation whose kit is being evaluated. The authors note that all of the work was performed independent of that author, using only the package insert.

J Mol Diagn 2006 8:312-319: The authors are affiliated with Genesis Genomics Inc and own stock options.

J Mol Diagn 2006 8:320-329: The authors are employees of Veridex LLC and some have stock options in Johnson & Johnson.

J Mol Diagn 2006 8:371-375: The authors are affiliated with Quest Diagnostics.

J Mol Diagn 2006 8:376-384: The authors received financial support from Pfizer Pharmaceutical and Elan Pharmaceutical.

SEE EXAMINATION ANSWER SHEET – NEXT PAGE

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CME Questions # 21-30

Examination Answer Sheet #3, Questions #21-30					
Answer	a	b	c	d	e
Question #21	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #22	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #23	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #24	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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Question #26	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #27	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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Question #29	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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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 14 days.**

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