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CME Questions # 11-20

(See February Examination Sheet for Questions #1-10)

11. Overexpression of the *HER2* (*ERBB2*) gene has prognostic and therapeutic implications. Based on the referenced Technical Advance article that evaluates *HER2* gene amplification using an automated fluorescence *in situ* hybridization (FISH) enumeration system, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:144-150]*

- HER2 protein is overexpressed in the majority of invasive human breast carcinomas.
- Overexpression of HER2 is rare in invasive lobular carcinoma.
- The most common mechanism of HER2 overexpression is gene amplification.
- HER2 overexpression is associated with better response rates to trastuzumab.
- The *HER2* gene is located on chromosome 17 and encodes a 185-kd transmembrane glycoprotein with intracellular tyrosine kinase activity.

12. FISH is a commonly used method for assessing *HER2* status. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:144-150]*

- The most widely used approaches to assess *HER2* status include immunohistochemistry and FISH.
- The study design involved duplicate slide sets of formalin-fixed, paraffin-embedded human breast tissue specimens that were prepared by hybridization of fluorescent probes prior to distribution to three separate laboratory sites; each set of slides was analyzed within 24 hours of receipt.
- Among the potential concerns when enumerating FISH signals via an automated system is that even thin tissue sections are three dimensional and often require focusing up and down through the tissue to determine accurate signal counts.
- Among specimens with informative results for both manual and automated methods, classification of results were concordant in 92.5% of slides tested, and when the intermediate range (as specified by the manufacturer) scanner data were excluded using the automated method, the concordance rate between automated and manual classification increased to 98.8%.
- The average time to obtain a result for the automated enumeration methods was significantly less than the time to obtain a result for the manual enumeration method.

13. Because of their low abundance, analysis of tyrosine phosphoproteins has been difficult. Based on the referenced article that describes an enrichment technique for identification of tyrosine phosphoproteins in cancer cells, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:169-177]

- Notable protein tyrosine kinases (PTKs) that are deregulated in human cancers include BCR-ABL, KIT, platelet-derived growth factor receptor (PDGFR), and nucleophosmin-anaplastic lymphoma kinase (NPM-ALK).
- The expression of NPM-ALK in anaplastic large cell lymphoma results from the t(2;5)(p23;q35) chromosomal rearrangement.
- Immobilized metal affinity chromatography (IMAC) is an approach that specifically isolates phosphotyrosine-containing peptides.
- Sodium orthovanadate treatment of cells improved enrichment of tyrosine phosphoproteins and their detection by liquid chromatography-tandem mass spectrometry.
- Peptides from proteins enriched by immunoprecipitation were more abundant than those enriched by immunoaffinity chromatography.

14. Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system. Based on the referenced article that identifies molecular biomarkers for MS, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9: 197-204]*

- a. There is currently no single definitive laboratory test for MS.
- b. The relative expression levels of 9 genes were determined using quantitative real-time polymerase chain reaction (Q-RT-PCR) and an optimal discriminatory ratio was found that separated control and MS individuals, in which the largest test ratio for the control individuals was less than the smallest test ratio for the MS individuals.
- c. *TAF11* was the most under-expressed gene in the majority of MS patients and was represented in all of the component ratios.
- d. The best discriminator between MS individuals and controls was derived from a 4-ratio combination of genes.
- e. Standard control genes such as glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) or β -actin (*ACTB*) were not employed because they showed some degree of statistically significant difference in microarray datasets between the subject groups.

15. MS is a demyelinating disease with a presumed autoimmune etiology. Based on the referenced article that identifies molecular biomarkers to discriminate MS individuals from subjects with other chronic diseases and from controls, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:197-204]*

- a. Epidemiologic data along with genetic linkage studies support the presence of a genetic contribution to susceptibility to autoimmune disease.
- b. In this study, MS patients were classified into relapsing remitting, primary progressive, secondary progressive, and pre-MS disease subtypes, but no scoring pattern could be identified with a specific sub-type.
- c. Common therapies for MS include β -interferon, copaxone, methotrexate, and prednisone. There were no significant differences between the various treatment groups, but the difference between each treatment group and the control group was significant.
- d. A scoring system was identified that discriminated between MS individual and subjects with other autoimmune diseases.
- e. Pre-MS patients received positive scores.

16. One of the factors contributing to diagnostic errors in surgical pathology is misidentification of patients' paraffin-embedded tissues. Based on the referenced article that describes a single nucleotide polymorphism (SNP) profiling assay to confirm the identity of patient tissues, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:205-213]

- a. SNPs occur approximately once every 100 to 300 base pairs in the human genome.
- b. The power of discrimination may exceed 10^{11} when using a combination of many short tandem repeat (STR) systems.
- c. Because the size of DNA fragments is limited in paraffin-embedded tissues, the SNP profiling assay has a significant advantage over STR-based kits.
- d. The accuracy of the ten-SNP profiling test described exceeded the power of discrimination of STR systems.
- e. An advantage of the SNP profiling assay over other methods currently used to exclude sample labeling errors is that it can be performed in any real-time PCR machine and does not require DNA sequencing equipment.

17. SNP amplification assays have been developed using DNA probes that form stable duplexes with single-stranded DNA targets, thus allowing short probes to be used for hybridization-based assays. Based on the referenced article, select the ONE statement regarding selection of SNP probes for the profiling assay that is NOT true: [See J Mol Diagn 2007 9:205-213]

- a. SNPs should ideally reside within a coding region of a gene.
- b. SNPs should be situated on different chromosomes.
- c. The minor (and major) allele frequency of the SNP should be approximately 0.5.
- d. One sequence specific to the human Y-chromosome was included to resolve male/female sample mix-ups.
- e. It is important to select SNPs on the basis of allele frequencies within the population studied.

18. The American College of Obstetricians and Gynecologists (ACOG) currently recommends Ashkenazi Jewish (AJ) population-based carrier screening for several inherited disorders. Based on the referenced article describing the development of a population- and disease-specific microarray for the detection of 15 different disorders that are prevalent in individuals of primarily AJ descent, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:228-236]*

- a. Of the three discrete groups of Jewish populations in the world today, Ashkenazi Jews account for approximately 90% of the Jews living in the United States.
- b. The American College of Medical Genetics (ACMG) currently recommends a panel of 23 *CFTR* mutations be offered to anyone who considers having children; however, the *CFTR* mutations prevalent in the AJ population are not represented in this panel.
- c. In addition to cystic fibrosis, ACOG currently recommends AJ population-based carrier screening for Tay-Sachs disease, Canavan disease, and familial dysautonomia.
- d. The authors developed a microarray panel that tests for 15 conditions representing the majority of the conditions found primarily in the AJ population except for cystic fibrosis.
- e. Several mutations in the selected disorders that are not prevalent in the AJ population were also included in the microarray panel.

19. Arrayed primer extension (APEX) genotyping has been previously applied to the detection of several inherited disorders. Based on the referenced article on an APEX microarray panel for conditions prevalent among AJ populations, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:228-236]*

- a. APEX technology combines a microarray-based assay with Sanger sequencing and is performed on a glass slide containing a two-dimensional array of 5'-immobilized oligonucleotides to which a PCR-amplified DNA sample is annealed.
- b. Oligonucleotide primers were designed according to the wild-type gene sequences for both the forward and reverse directions.
- c. For each mutation, four points were available for data analysis.
- d. APEX failure is mainly due to self-annealing secondary structures that result in self-priming and extension or failure to hybridize.
- e. To enable sufficient fragmentation of PCR products with uracil N-glycosylase (UNG), it is necessary to substitute 40% of dTTPs with dUTPs.

20. Hereditary hemorrhagic telangiectasia (HHT) is a vascular dysplasia. Based on the referenced article describing clinical and analytical sensitivities in HHT testing, select the ONE statement that is NOT true: [See J Mol Diagn 2007 9:258-265]

- a. HHT is characterized phenotypically by telangiectases and arteriovenous malformations.
- b. The frequency of HHT is reported to be about 0.01%.
- c. The endoglin (*ENG*) and activin receptor-like kinase 1 (*ACVRL1*) genes have been reported to cause HHT in an autosomal dominant fashion if mutated.
- d. Temperature gradient capillary electrophoresis (TGCE), a relatively new technique that allows for screening of heteroduplexes, can detect large deletions and duplications in addition to most point mutations in an amplicon.
- e. Several polymorphisms located in introns and towards the ends of amplicons were missed by TGCE.

***Disclosures:**

J Mol Diagn 2007 9:144-150: Support for this study was provided by Abbott Laboratories, where one of the authors is affiliated.

J Mol Diagn 2007 9:197-204: This work was supported in part by a grant from the National Institutes of Health to ArthroChip. Two of the authors are the sole owners of ArthroChip. Four of the authors are inventors of a patent that has been submitted by Vanderbilt University.

J Mol Diagn 2007 9:228-236: This work was supported in part by funds from Reprogenetics Research, Inc, where one of the authors is affiliated. One of the authors is affiliated with Asper Biotech Ltd.

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	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #12	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #13	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #14	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #15	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #16	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #17	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #18	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #19	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Question #20	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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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.
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