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American Society for Investigative Pathology *and the*
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ANSWERS for CME Questions # 1-8
1b, 2b, 3e, 4d, 5d, 6c, 7e, 8b

1. Rapid and economical molecular diagnosis of fungal infection is an unmet need in clinical medicine. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010 12:17-19; DOI: 10.2353/jmoldx.2010.090173 and J Mol Diagn 2010 12:91-101; DOI: 10.2353/jmoldx.2010.090085; the authors of the referenced articles did not disclose any relevant financial relationships.]

a. Fungal infection affects immunocompromised hosts and patients hospitalized with severe underlying diseases. **This statement is TRUE. The types of fungal infection that occur in the patients vary according to the type of underlying disease of the host, as well as with their epidemiological exposure. Patients with neutropenia, for example, are at heightened risk for invasive filamentous fungal infections, whereas patients in the intensive care unit who have indwelling intravascular access catheters are at increased risk for infections by yeasts.**
b. The best method for rapid detection, identification, and quantification of fungal infections from clinical samples is direct culture. **This statement is NOT TRUE. Many fungal species do not culture efficiently or require unacceptably long incubation times.**
c. Although histopathology is useful in confirming the presence of many types of fungal infection, even the most highly skilled infectious disease pathologist has limitations in evaluating the morphological differentiation of many fungi that cause invasive disease. **This statement is TRUE. For example, it is usually not possible to definitively differentiate the types of hyaline septate molds (eg, *Aspergillus* versus *Fusarium* versus *Pseudallescheria boydii*) that may be responsible for the infection.**
d. Fluorescence *in situ* hybridization using peptide nucleic acid probes is in clinical use for identification of *Candida albicans* and *C. glabrata*. **This statement is TRUE. However, this tool requires an initial culture step to increase fungal titer to detectable levels and is limited in the number of species it can identify.**
e. PCR-based strategies may meet the challenges of fungal diagnostics. **This statement is TRUE. Challenges for PCR-based studies include low cell numbers, potentially <1 cell/ml sample, lysing fungal walls, and the similarity in ribosomal DNA sequences to human.**

2. PCR strategies using panfungal primers that complement conserved regions of ribosomal DNA but span the variable internal transcribed spacer regions (ITS1 and ITS2) can detect any and all fungal species in a single reaction. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010 12:17-19; DOI: 10.2353/jmoldx.2010.090173 and J Mol Diagn 2010 12:91-101; DOI: 10.2353/jmoldx.2010.090085; the authors of the referenced articles did not disclose any relevant financial relationships.]

a. Amplicons from panfungal primers are often sequenced to identify species. **This statement is TRUE. Sequencing is performed using standard, automated capillary sequencing, pyrosequencing, or sequencing-grade microarrays.**
b. Restriction enzyme analysis of the amplicon provides precise identification of the base composition of the amplified sequences. **This statement is NOT TRUE. Restriction enzyme analysis provides adequate, but not precise, identification of the base composition of the amplified sequences.**
c. Electrospray mass spectroscopy may be used to identify the base composition of amplified sequences. **This statement is TRUE. Electrospray mass spectroscopy provides precise identification of the base composition of amplified sequences.**
d. Repetitive element PCR (REP-PCR), a version of random amplification of polymorphic DNA (RAPD) in which primers target repetitive sequence elements, has been used for fungal identification. **This statement is TRUE. However, REP-PCR requires pure cultures as the starting material, which is useful in some applications but is not an acceptable precondition for a clinical fungal diagnostic tool.**

e. After panfungal amplication, specific probes may be used to identify the specific fungal species. **This statement is TRUE. Probes may be used either with standard hybridization after PCR, during amplification, or with hybridization-based FRET probes.**

3. Species-specific PCR may be used to identify fungal species in clinical samples. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010 12:17-19; DOI: 10.2353/jmoldx.2010.090173 and J Mol Diagn 2010 12:91-101; DOI: 10.2353/jmoldx.2010.090085; the authors of the referenced articles did not disclose any relevant financial relationships.]

a. Species-specific PCR is more sensitive that panfungal PCR. **This statement is TRUE. Species-specific PCR does not require further sequencing or identification of the product.**
b. *Candida* and *Aspergillus* species are good candidates for species-specific PCR identification. Species must be seen with relative frequency to be identified by species-specific PCR. **This statement is TRUE. Species that are certain to be seen with reasonable frequencies can be detected by species-specific PCR.**
c. Species-specific PCR requires that multiple assays are run for each sample. **This statement is TRUE. Running an assay for each primer set increases both cost and labor.**
d. Multiplexing could limit the number of assays run for each sample. **This statement is TRUE. Multiplexing, however, is associated with reduced sensitivity.**
e. Species-specific PCR is preferred to identify fungal infection of unknown species. **This statement is NOT TRUE. Many clinical samples will have novel species that may be missed by species-specific PCR.**

4. High resolution melt (HRM) analysis may be used to diagnose fungal infection. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010 12:17-19; DOI: 10.2353/jmoldx.2010.090173 and J Mol Diagn 2010 12:91-101; DOI: 10.2353/jmoldx.2010.090085; the authors of the referenced articles did not disclose any relevant financial relationships.]

a. In HRM analysis, the shape of the melting curve of an amplicon is used to identify fungal species. **This statement is TRUE. In HRM, short, regional sequences are denatured to form single-stranded regions, which release double-stranded DNA-binding fluorescent dyes before reaching the temperature at which the entire amplicon denatures. This influences the shape of the melt curve and generates nuances that reflect species-specific sequence differences.**
b. The HRM approach is likely to prove cost-effective. **This statement is TRUE. HRM does not require fluorophore-labeled probes or more extensive postamplification analysis.**
c. The ITS1-ITS2 domain has sufficient diversity among clinically important fungal species to allow their discrimination by HRM analysis. **This statement is TRUE. The average difference between species was approximately 25 base changes per hundred, ranging from 2 to 48.**
d. As HRM melt curves are not reproducible, standards must be run with every reaction. **This statement is NOT TRUE. HRM analysis generates melt curves that are reproducible over time, and a laboratory-specific database of reference species could be generated, stored, and used in the melt calculation without having to rerun standards every time.**
e. HRM analysis is capable of distinguishing all clinically important species of *Candida*, even if present at very limiting initial template concentrations. **This statement is TRUE. This is accomplished without the need for heteroduplex formation or internal control templates, which are needed to identify point mutations.**

5. Duchenne (DMD) and Becker muscular dystrophies (BMD) are caused by mutations in the dystrophin gene. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010 12:65-73; DOI: 10:2352/jmoldx.2010.090095; the authors of the referenced article did not disclose any relevant financial relationships.]

a. The DMD gene spans 2.2 million base pairs of genomic DNA on the X chromosome. **This statement is TRUE. The 14-kb transcript encodes a full-length protein (dystrophin) of 427 kDa (isoform Dp427m).**
b. Mutations in dystrophin are X-linked recessive. **This statement is TRUE. Greater than 99% of DMD and BMD cases are in males.**
c. The most common dystrophin mutations are large intragenic deletions or duplications. **This statement is TRUE. These deletions or duplications often encompass more than one exon.**
d. Of dystrophin mutations, more than half are point mutations that occur outside of mutation/duplication “hot spots.” **This statement is NOT TRUE. Only 15% to 25% of dystrophin mutations occur outside of mutation/duplication “hot spots.”**
e. The major group of point mutations are premature stop codons. **This statement is TRUE.**

6. Mutation detection is important for both genetic counseling of DMD and BMD patients' families and determining therapeutic options of mutation-specific treatments. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010 12:65-73; DOI: 10:2352/jmoldx.2010.090095; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Familial muscle biopsies are often not available because the affected family member is deceased. **This statement is TRUE. Muscle biopsy is often used to diagnose DMD and BMD.**
b. Mutation diagnosis for DMD mutations is often used for carrier testing. **This statement is TRUE. Mutation diagnosis is also used for prenatal screening.**
c. The first half of the C terminus and the cysteine-rich D-domain contain 80% of the known mutations of dystrophin. **This statement is NOT TRUE. The first half of the C terminus and the cysteine-rich D-domain (amino acid residues 3080-3408) are highly conserved regions of dystrophin. Deletions or chain-terminating nonsense mutations involving the D-domain usually result in DMD.**
d. It is estimated that mRNA or comparative genome hybridization (CGH) analyses are necessary to diagnose about 4% of all DMD/BMD patients. **This statement is TRUE. Mutations were found in 121 DMD-BMD patients out of 153 DNA samples tested. Considering that 153 DNA samples correspond to 20% of all patients that show no deletions or duplications, about 20% of patients cannot be diagnosed by DNA analysis alone.**
e. The DNA-based mutation screening strategy is suitable for high-throughput applications in patients for which mRNA is unavailable. **This statement is TRUE. With the availability of more sensitive methods of DNA detection (ie multicolor fluorescence), there is the possibility to pool dozens of samples with further reduction of costs.**

7. Multiplex RT-PCR can be used to differentiate influenza A, influenza B, and respiratory syncytial virus (RSV) A/B. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010 12:74-81; DOI: 10.2353/jmoldx.2010.090095; the authors of the referenced article did not disclose any relevant financial relationships.]

a. In the mid-1990s, RT-PCR replaced tissue culture as the gold standard for respiratory virus diagnosis. **This statement is TRUE. RT-PCR is significantly more sensitive and highly specific for the detection of influenza and RSV.**
b. Automated platforms have been developed to encompass nucleic acid extraction, RT-PCR amplification, and detection. **This statement is TRUE. The goals of these automated platforms have been to decrease technician time to only a few minutes, to make the assay time only 2 to 3 hours (or less), to decrease cost, and to keep the footprint of the equipment as small as possible.**
c. In 2008, the US Food and Drug Administration (FDA) approved two multiplex RT-PCR assays for the detection of influenza A, influenza B, and RSV A/B. **This statement is TRUE. One multiplex RT-PCR assay is capable of detecting 9 viruses and a combined 11 subtypes for three of these viruses, is an open format assay, which increases the chance for contamination, and requires approximately 8 hours for completion as well as the use of a flow cytometer. The other approved multiplex RT-PCR assay is semi-automated and is capable of simultaneously detecting influenza A, influenca B, and RSV and takes approximately 3 hours, excluding extraction time.**
d. In September 2008, the FDA approved a series of singleplex RT-PCR assays that are capable of typing influenza viruses as A or B and further subtyping influenza A viruses as H1, H3, or H5. **This statement is TRUE. However, the reagents for these assays are only readily available to members of the CDC's Laboratory Response Network (LRN).**
e. The authors of the referenced article developed semi-automated and fully-automated multiplex RT-PCR assays capable of detecting influenza A, influenza B, and RSV A/B with a competitive protein internal control. **This statement is NOT TRUE. The assay by Beck et al has a non-competitive RNA internal control (MS2 RNA phage).**

8. Infections caused by a novel influenza virus [H1N1 swine origin influenza virus (S-0IV)] began to be recognized in March 2009. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010 12:74-81; DOI: 10.2353/jmoldx.2010.090095; the authors of the referenced article did not disclose any relevant financial relationships.]

a. In the current study, 57 out of 100 previously tested nasopharyngeal swab samples were determined to be positive for influenza A, influenza B, or RSV by tissue culture. **This statement is TRUE. The tissue culture results were used as the true result for sensitivity and specificity calculations of the multiplex RT-PCR assays.**
b. The authors developed and validated both semiautomated and fully automated multiplex real-time RT-PCR assays for the detection of influenza A, influenza B, and RSV from nasopharyngeal specimens that could distinguish influenza A infections of all subtypes from influenza B and RSV infections without a single case of cross-reaction among 23 common respiratory pathogens. **This statement is NOT TRUE. There was one minor intermittent cross-reaction to adenovirus at $>10^7$ $TCID_{50}$/ml.**

c. Precision was determined by calculating the coefficient of variation of the Ct values reported for the positive controls on each run over a 4-month time period. **This statement is TRUE. In addition, the mean and SD in Tm of each analyte were calculated by analyzing all data obtained from clinical testing during a 2-week period.**

d. Viruses from various hosts, collection years, and geographic locations were analyzed on the semiautomated and fully autmoated platforms. **This statement is TRUE. The melting temperature (Tm) ranges reported for influenza A, influenza B, and RSV viruses by Beck et al are therefore highly representative of their true ranges for these platforms.**

e. Both the semiautomated and fully automated assay formats developed by Beck et al have rapid sample turnaround times with limited technician time. **This statement is TRUE. Both platforms require approximately 20 to 40 minutes of technician time and can return results in 2.5 to 3.5 hours for 24 samples.**

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ANSWERS for CME Questions # 9-17
9c, 10d, 11e, 12a, 13b, 14e, 15a, 16b, 17c

9. The establishment of standards and reference materials to support the application of molecular diagnostics to patient management has lagged advances in molecular infectious disease testing. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:133-143; DOI: 10.2352/jmoldx.2010.090067; Roberta Madej was an employee of Roche Molecular Diagnostics (Pleasanton, CA); Jack Davis and Stan Kwang are employees of the Quality Systems Divisions of Bio-Rad Laboratories (San Ramon, CA and Irvine, CA, respectively); Emmanuel Labourier is an employee of Asuragen, Inc (Austin, TX); George J. Schneider is an employee of Research and Development, Abbot Molecular Inc. (Des Plaines, IL); the remaining author of the referenced article did not disclose any relevant financial relationship.]

a. Many of the early molecular assays lacked adequate reproducibility and comparability, as illustrated in proficiency testing reports. **This statement is TRUE. This inadequacy was attributed to the abundant use of laboratory-developed protocols, inconsistent training, limited utilization of standardized industry-produced assays, and the absence of adequate universal standards for test development.**
b. Patients are being monitored for increasing lengths of time, and consistent tests are required to compare changes in values over time. **This statement is TRUE. Current clinical practice recommendations direct serial specimens of patients to be optimally tested with the same assay each time they are submitted.**
c. Practitioners have an increasing ability to demand the use of specific laboratories and tests, ensuring assay consistency. **This statement is NOT TRUE. Practitioners have a decreasing ability to demand the use of specific laboratories and tests.**
d. Many non-specialist treatment providers have limited knowledge of practice standards. **This statement is TRUE. There are an increasing number of non-specialist treatment providers.**
e. The requirement for translatable quantification among different molecular tests relies on universally available, robust standard reference materials for test developers, manufacturers, and proficiency test providers. **This statement is TRUE. The utility of quantitative molecular diagnostics for patient management depends on the ability to relate patient results to prior results or to absolute values in clinical practice guidelines.**

10. The World Health Organization (WHO) International Standard (IS) for hepatitis C virus (HCV) was the first global standard for the calibration and characterization of quantitative molecular viral assays. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:133-143; DOI: 10.2352/jmoldx.2010.090067; Roberta Madej was an employee of Roche Molecular Diagnostics (Pleasanton, CA); Jack Davis and Stan Kwang are employees of the Quality Systems Divisions of Bio-Rad Laboratories (San Ramon, CA and Irvine, CA, respectively); Emmanuel Labourier is an employee of Asuragen, Inc (Austin, TX); George J. Schneider is an employee of Research and Development, Abbot Molecular Inc. (Des Plaines, IL); the remaining author of the referenced article did not disclose any relevant financial relationship.]

a. The first WHO IS HCV standard was a preparation of a HCV genotype 1a high-titered plasma unit diluted into cryosupernatant and lyophilized. **This statement is TRUE. The preparation was tested with two other candidate materials in a global collaborative study in 22 laboratories during 1996.**
b. Most of the data for the first WHO IS HCV standard were generated by testing endpoint dilutions of the candidate standards using qualitative traditional (non-real-time) amplification assays. **This statement is TRUE. However, quantitative data submitted by participants were included in the analysis.**
c. The first WHO HCV IS (96/790) was assigned the value of 10^5 International Units (IU)/ml (5 $\log_{10}$ IU/ml) lyophilized in 0.5 ml ampoules. **This statement is TRUE. The IU is considered absolute, and consistent with WHO policies, there are no units of uncertainty associated with this or subsequent replacements.**
d. The first HCV IS, developed in 1997, was replaced by one subsequent standard in 2006. **This statement is NOT TRUE. Since 1997, the first HCV IS has been replaced by two subsequent standards: the second WHO**

HCV IS (96/798) in 2003, which was a second lyophilization preparation of the original material, and most recently a new material prepared and tested in 2007.

e. For the most recent HCV IS, new materials were commissioned and a full global replacement study directed by the National Institute for Biological Standards and Control (NIBSC) was performed. **This statement is TRUE. Most assays were quantitative, although data testing the limiting dilutions of the materials with qualitative molecular assays were also included. Additionally, real-time amplification assays not present in the first study over ten years earlier had become common and were reflected in the submission of data.**

11. Consultation meetings with the WHO and working groups such as Standardization of Genome Amplification Techniques (SoGAT) have provided recommendations for the desired specifications of standards for quantitative molecular diagnostics. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:133-143; DOI: 10.2352/jmoldx.2010.090067; Roberta Madej was an employee of Roche Molecular Diagnostics (Pleasanton, CA); Jack Davis and Stan Kwang are employees of the Quality Systems Divisions of Bio-Rad Laboratories (San Ramon, CA and Irvine, CA, respectively); Emmanuel Labourier is an employee of Asuragen, Inc (Austin, TX); George J. Schneider is an employee of Research and Development, Abbot Molecular Inc. (Des Plaines, IL); the remaining author of the referenced article did not disclose any relevant financial relationship.]

a. A prominent group in discussions regarding performance specifications for quantitative molecular diagnostics IS has been the Industrial Liaison Committee (ILC). **This statement is TRUE. The ILC is an organization of molecular assay manufacturers working towards the availability of universally accepted reference standards with a specific interest in developing standards to serve the development of quantitative molecular diagnostic assays used in patient testing.**
b. SoGAT meetings have discussed the establishment of reference materials similar to clinical samples and quantification by methods independent of the current diagnostic testing methods. **This statement is TRUE. These meetings have also discussed preparations with concentrations adequate to evaluate the entire expected clinical range, dilution protocols and published dilution recovery expectations, and stability testing.**
c. For commercial HCV and HIV molecular quantitative assays where international standards have been available, variability has decreased significantly since the first generation. **This statement is TRUE. In contrast, a standard is not available for Epstein-Barr virus (EBV). A recent survey of 28 laboratories examining the variability of EBV assays across various viral load concentrations, sample types, and assay platforms demonstrated that significant variation still exists.**
d. There is still significant variability for EBV assays, strongly suggesting a need for assay calibration to a universally-accepted reference standard. **This statement is TRUE. High levels of variation in results was observed between samples containing virally-infected cellular material, suggesting sample preparation methodology, such as DNA extraction, needs further improvements. Variability was also significantly higher in inter-laboratory comparisons versus intra-laboratory comparisons.**
e. The established standard for human cytomegalovirus (HCMV) assays in the United States, Europe, and Canada has minimized assay variability. **This statement is NOT TRUE. HCMV assays have demonstrated comparable problems as EBV. Among 33 laboratories testing a panel of seven constructed samples and three patient-derived samples, 57.6% of quantitative results were within the range of ± 0.5 $\log_{10}$ of expected results. Though the authors attributed the variation to several factors, they suggested that a primary measure towards resolution would be the establishment of an international reference standard.**

12. Synthetic nucleotides may provide an alternative HCV standard. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:133-143; DOI: 10.2352/jmoldx.2010.090067; Roberta Madej was an employee of Roche Molecular Diagnostics (Pleasanton, CA); Jack Davis and Stan Kwang are employees of the Quality Systems Divisions of Bio-Rad Laboratories (San Ramon, CA and Irvine, CA, respectively); Emmanuel Labourier is an employee of Asuragen, Inc (Austin, TX); George J. Schneider is an employee of Research and Development, Abbot Molecular Inc. (Des Plaines, IL); the remaining author of the referenced article did not disclose any relevant financial relationship.]

a. Limiting dilution viral culture is required to propagate and measure HCV. **This statement is NOT TRUE. HCV cannot be propagated and measured by independent methods such as limiting dilution viral culture.**
b. *In vitro*-generated HCV RNA fits the characteristics for an HCV standard. **This statement is TRUE. A well-characterized, *in vitro*-generated HCV RNA transcript, quantified by physical-chemical assays to a primary phosphate standard, was available for testing.**
c. Four nucleic acid testing methods were studied to determine the feasibility of using synthetic RNA as an HCV standard. **This statement is TRUE. Six to eight replicates of four to six linear points of each material were tested in the manufacturers'laboratories.**
d. Synthetic targets could be recovered and quantified with equal or less variability than the biological materials. **This statement is TRUE. Synthetic materials demonstrated full utility based on recovery, range of linearity, and variation.**
e. A well-characterized and independently-tested synthetic nucleic acid material could serve as the constant in an environment in which reference methods are not available. **This statement is TRUE. If stable synthetic materials could be established together with the international biologic standard and the relationship between the two quantified for relevant technologies, the synthetic material could be tested with each replacement lot of the biologic standard.**

13. The National Institute of Standards and Technology (NIST) is developing a standard reference material (SRM) for HCMV. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:133-143; DOI: 10.2352/jmoldx.2010.090067; Roberta Madej was an employee of Roche Molecular Diagnostics (Pleasanton, CA); Jack Davis and Stan Kwang are employees of the Quality Systems Divisions of Bio-Rad Laboratories (San Ramon, CA and Irvine, CA, respectively); Emmanuel Labourier is an employee of Asuragen, Inc (Austin, TX); George J. Schneider is an employee of Research and Development, Abbot Molecular Inc. (Des Plaines, IL); the remaining author of the referenced article did not disclose any relevant financial relationship.]

a. HCMV is present both intracellularly and in the plasma. **This statement is TRUE. A standard that is free of biologic material may be an appropriate reference for the development of several types of biologic standards.**
b. The NIST standard will consist of pure HCMV RNA. **This statement is NOT TRUE. The NIST standard will consist of pure HCMV DNA.**
c. The HCMV SRM should be suitable as a calibrant for quantitative PCR assays of HCMV viral load. **This statement is TRUE. It should also be used as a tool to standardize various nucleic acid amplification technology (NAT) assay reagents and calibrants produced by manufacturers of diagnostics or laboratory-developed protocols.**
d. Although quantitative real-time PCR is becoming the method of choice for HCMV quantification, there are issues such as DNA sequence diversity among targets used for quantification that can result in false negatives. **This statement is TRUE. There is a proliferation of assays with different target sequences in the HCMV genome. In a recent survey, 10 different open reading frame targets were used, glycoprotein B most frequently.**
e. The HCMV standard reference material will consist of pure genetic material from a bacterial artificial chromosome (BAC) of the HCMV Towne strain. **This statement is TRUE. The Towne BAC is stable and can provide large quantities of consistent viral DNA, whereas cultured virus, especially from laboratory strains of HCMV, can produce truncated genomes. Genome size consistency is important for calculation of genome copy number.**

14. Tumor-specific chromosome abnormalities identified in malignant cells have provided insight into the mechanisms of tumorigenesis as well as clinically applicable prognostic disease markers. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:144-146; DOI: 10.2352/moldxj.2010.09023 and J Mol Diagn 2010, 12:184-196; DOI: 10.2352/moldxj.2010.090118; the authors of the referenced articles did not disclose any relevant financial relationships.]

a. "Smash and smear" cytogenic techniques revealed chromosome number and morphology in malignant cells to be different from those of healthy cells. **This statement is TRUE. These observations were made even before the correct *Homo sapiens* chromosome number was determined in 1956.**
b. In the early 1970s, chromosome banding techniques were used to identify recurrent chromosomal abnormalities. **This statement is TRUE. However, the universal application of chromosome analysis in clinical oncology has been hampered by the difficulty involved in obtaining metaphases from most types of tumor cells in the laboratory and the relatively low resolution of chromosome banding techniques even when metaphases are successfully obtained.**
c. Fluorescence *in situ* hybridization (FISH) has made the evaluation of structural and numerical chromosomal alterations an integral part of diagnosis and prognosis of malignancies. **This statement is TRUE. DNA-based FISH probes allow for a higher resolution analysis than traditional karyotyping.**
d. Array-based testing methods merge the high resolution locus-specific DNA-based analysis of FISH probes with a global genome view similar to that obtained by conventional karyotyping. **This statement is TRUE. The various array platforms currently used for clinical oncology analyses derive results by comparing hybridization intensity values of malignant (test) and normal (reference) DNA samples.**
e. In this study, Hagenkord et al validated a deletional mutant array platform for clinical use in the prognosis of newly diagnosed chronic lymphocytic leukemia (CLL). **This statement is NOT TRUE. In this study, a single nucleotide polymorphism (SNP) array platform was validated for clinical use in the prognosis of newly diagnosed CLL.**

15. Array-based karyotyping is well-suited for prognostic marker assessment of CLL. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:144-146; DOI: 10.2352/moldxj.2010.09023 and J Mol Diagn 2010, 12:184-196; DOI: 10.2352/moldxj.2010.090118; the authors of the referenced articles did not disclose any relevant financial relationships.]

a. The clinically relevant genomic lesions in CLL are balanced translocations, rather than chromosomal gains and losses, which would be undetectable using current array technology. **This statement is NOT TRUE. The clinically relevant genomic lesions in CLL are chromosomal gains and losses, rather than balanced translocations, which would be undetectable using current array technology.**
b. Fresh CLL samples with verifiable high tumor burden can readily be obtained from peripheral blood. **This statement is TRUE. Fresh CLL samples can also be obtained from bone marrow specimens.**

c. Prognostic markers in CLL are commonly clinically assessed because it is a microscopically homogenous and clinically heterogeneous disease. **This statement is TRUE. Patients with morphologically similar abnormal lymphocytes can have dramatically different disease courses.**
d. Evaluation of recurrent chromosomal abnormalities with prognostic significance in newly diagnosed CLL has become one of the major criteria for patient risk assessment. **This statement is TRUE. This need makes it imperative that tumor genome markers be evaluated completely and accurately.**
e. Recent studies have revealed that conventional cytogenetics and FISH panels are inadequate for evaluation of the CLL tumor genome. **This statement is TRUE. However, the vast majority of newly diagnosed cases of CLL are still evaluated using these methods.**

16. There may be as many as 3 million single nucleotide polymorphisms (SNPs) in the human genome. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:162-168; DOI: 10.2352/jmoldx.2010.090084; the authors of the referenced article did not disclose any relevant financial relationships.]

a. An SNP is a single nucleotide variation at a specific location in the genome. **This statement is TRUE. SNPs may be used as genetic markers to determine an individual's susceptibility to various diseases.**
b. By definition, a single nucleotide variation must be found in at least 2% of the population to be called an SNP. **This statement is NOT TRUE. SNPs are single nucleotide variations that are found in more than 1% of the population.**
c. SNPs have been used as molecular markers to evaluate disease processes. **This statement is TRUE. SNPs have also been used to predict patients' drug responses.**
d. To identify SNPs, PCR amplification of a desired SNP-containing region is performed initially to introduce specificity and increase the number of allele-specific molecules. **This statement is TRUE. Afterwards, amplified DNA fragments containing a specific SNP are measured by a device based on mass or another biochemical property.**
e. The unique features of surface-enhanced laser desorption and ionization-time of flight (SELDI-TOF) mass spectrometry make it the most plausible device for genotype testing of SNPs because it can rapidly and efficiently isolate the targeted oligonucleotides from other reaction reagents, therefore greatly improving the detection process. **This statement is TRUE. With SELDI-TOF mass spectrometry, it is possible to pre-select a chip (based on the properties of target analytes), enrich a target molecule, remove unwanted elements such as salt, and then detect it using a mass spectrometer.**

17. Warfarin is an anticoagulant that disrupts the process of vitamin K recycling. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:162-168; DOI: 10.2352/jmoldx.2010.090084; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Vitamin K is an essential cofactor for the post-translational modification of several clotting factors. **This statement is TRUE. Vitamin K is essential in the modification of Factors II, VII, IX and X and the anticoagulant proteins C and S.**
b. Therapeutic warfarin leads to low clotting potential in patients. **This statement is TRUE. These patients have a lower propensity for thrombotic disorders.**
c. Warfarin enhances the function of vitamin K epoxide reducatase 1 (VKORC1). **This statement is NOT TRUE. Warfarin inhibits the function of VKORC1, which is responsible for regeneration and recycling of vitamin K. Suppression of VKORC1 results in a reduction of endogenous vitamin K and a subsequent reduction of vitamin K-dependent clotting factors.**
d. *VKORC1* variants exhibit different sensitivities to warfarin. **This statement is TRUE. Patients who are found to have the genotype AA, for the SNP *VKORC1 3673G>A* in the promoter region, have a lower amount of VKORC1 and therefore typically require a lower warfarin dose than the average patient. In contrast, those with GG genotype are resistant to warfarin, typically requiring a greater dose of the drug to achieve desired therapeutic effect. Other patients carry an AG genotype.**
e. Warfarin drug effect is primarily removed when it is converted to a hydroxylated metabolite by hepatic microsomal enzymes (cytochrome P-450). **This statement is TRUE. The cytochrome P-450 2C9 (CYP2C9) isozyme appears to be the principal form of human liver P-450, and it controls the drug activity of warfarin. The variant alleles, particularly variant *CYP2C9*2* and *CYP2C9*3*, result in decreased hydroxylation of warfarin, thus reducing the rate of warfarin clearance.**

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ANSWERS for CME Questions # 18-26
18b, 19d, 20e, 21c, 22a, 23e, 24d, 25a, 26c

18. Chronic granulomatous disease (CGD) is caused by defects in the respiratory burst NADPH oxidase complex in phagocytes. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:269-271; DOI: 10.2353/jmoldx.2010.100004 and J Mol Diagn 2010, 12:368-376; DOI: 10.2353/lmoldx.2010.09147; Aspects of high resolution melting are licensed by the University of Utah to Idaho Technology; Joshua D. Bagnato is an employee of Idaho Technology;. Carl T. Wittwer has an equity interest in Idaho Technology. None of the other authors disclosed any relevant financial relationships.]

a. Intracellular bactericidal defects in phagocytes lead to recurrent, severe bacterial and fungal infections. **This statement is TRUE. These infections often occur in the skin, lungs, visceral organs, and bones.**
b. In patients with CGD, granuloma formation occurs at sites distal from infections. **This statement is NOT TRUE. In patients with CGD, granuloma formation often occurs at the site of infection.**
c. Most cases of CGD are X-linked and caused by mutations in *CYBB.* **This statement is TRUE. X-linked *CYBB* mutations account for 65% to 70% of cases, and ~25% arise from mutations in *NCF1.***
d. Screening for CGD is usually performed by functional assays such as the neutrophil respiratory burst assay. **This statement is TRUE. This assay is performed by flow cytometry.**
e. It is not possible to distinguish autosomal recessive from X-linked CGD by flow cytometric analysis. **This statement is TRUE. Different mutations in autosomal recessive genes may result in either absent or decreased oxidase activity, similar to X-linked disease.**

19. CGD is a rare genetic disorder affecting ~1/200,000 individuals. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:269-271; DOI: 10.2353/jmoldx.2010.100004 and J Mol Diagn 2010, 12:368-376; DOI: 10.2353/lmoldx.2010.09147; Aspects of high resolution melting are licensed by the University of Utah to Idaho Technology; Joshua D. Bagnato is an employee of Idaho Technology;. Carl T. Wittwer has an equity interest in Idaho Technology. None of the other authors disclosed any relevant financial relationships.]

a. The *CYBB* gene was found to be responsible for X-linked CGD transmission via positional cloning. **This statement is TRUE. *CYBB* encodes a 91-kD glycoprotein that functions as a phagocyte oxidase subunit. The discovery of *CYBB* identified for the first time a gene responsible for a human genetic disease based on its chromosomal location without reference to a specific protein product.**
b. Two new genetic subgroups of CGD have autosomal recessive mutations in *RAC2* and *NCF4*. **This statement is TRUE. *RAC2* and *NCF4* encode the two NADPH oxidase partners *rac2* and $p40^{phox}$, respectively.**
c. Mutations in the *CYBB* gene are involved in the most frequent CGD form (comprising 60% of CGD cases). **This statement is TRUE. Strategies used to detect these mutations include single-strand conformation polymorphism (SSCP) analysis, denaturing high-pressure liquid chromatography (DHPLC), and direct DNA sequencing of all 13 exons and adjacent splice sites.**
d. Hill and colleagues analyzed the *CYBB* gene using fluorescence *in situ* hybridization. **This statement is NOT TRUE. Hill and colleagues analyzed the *CYBB* gene using high-resolution melting (HRM) technology. This method has previously been developed for common genetic diseases such as cystic fibrosis.**
e. *CYBB* is a good candidate for genetic analysis because polymorphisms are rare and exon sizes are compatible with the method. **This statement is TRUE. *CYBB* is also a good candidate for HRM genetic analysis because most of the mutations are missense or nonsense mutations or small insertions or deletions; only rarely are large mutations, such as deletion of the entire gene, detected.**

23. The lack of a low cost, minimally invasive diagnostic assay to monitor disease activity hinders the development of new therapies for MS. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010 12:312-319; DOI: 10.2353/jmoldx.2010.090170; Howard B. Urnovitz, Julia Beck, and Ekkehard Schütz are employees of Chronix Biomedical; William M. Mitchell is an independent Board of Directors member; Mario Clerici and William M. Mitchell serve on the Chronix Medical Advisory Committee; none of the other authors disclosed any relevant financial relationships.]

a. High per-patient costs make it prohibitively expensive to study the comparative effectiveness of a treatment, prevention, or diagnostic regimen as it transitions from clinical trial to the larger venue of clinical practice. **This statement is TRUE. The high per-patient cost of Gd-MRI limits the number of patients studied in randomized controlled clinical trials as well as the rate at which important questions can be tested.**
b. The cost for maximizing disease control in clinical practice has adverse economic consequences for the uninsured patient. **This statement is TRUE. High cost also has societal effects on the health insurance industry as well as local, state, and federal governments.**
c. Several repeat sequences, such as the L1 family of LINE elements, are consistently different in all MS patients as compared with the normal database. **This statement is TRUE. Coding genes also distinguish MS and its clinical activity.**
d. Mass sequencing and assembly (MSA) technologies can be used to identify and quantify unique DNA motifs in the serum of patients with MS. **This statement is TRUE. The differential frequencies of specific DNA motifs in patients with MS may be translated into a rapid serum-based diagnostic assay for MS and used to assess its clinical activity.**
e. MSA technologies identify differential lipid contents in the blood of MS patients. **This statement is NOT TRUE. MSA technology provides sequence data that can differentiate frequencies of specific DNA motifs in patients with MS.**

24. L1 elements (LINES) and human endogenous retroviruses (HERVs) encode a reverse transcriptase that provides an RNA intermediate for new chromosomal integration sites. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010 12:312-319; DOI: 10.2353/jmoldx.2010.090170; Howard B. Urnovitz, Julia Beck, and Ekkehard Schütz are employees of Chronix Biomedical; William M. Mitchell is an independent Board of Directors member; Mario Clerici and William M. Mitchell serve on the Chronix Medical Advisory Committee; none of the other authors disclosed any relevant financial relationships.]

a. LINES are the most active autonomous elements of the human genome. **This statement is TRUE. LINES are estimated to be present in >500,000 copies.**
b. Only 30-60 LINES are active transposing elements. **This statement is TRUE. This number is limited because of truncations associated with reverse transcriptase dissociation from its RNA template as well as various disruptive mutations.**
c. LINES have been postulated to be derived from LINE-mediated retrotransposition of retroviral mRNA. **This statement is TRUE. This hypothesis is based on the similarity of LINE-generated pseudogenes and the HERV-W family of retroviruses.**
d. L1 retrotransposons are not sensitive to siRNAs. **This statement is NOT TRUE. L1 retrotransposons are sensitive to regulation by siRNAs, suggesting the possibility of pharmaceutically targeting these elements.**
e. LINES might have some role in the dynamic equilibrium of auto-reactive T lymphocytes. **This statement is TRUE. Auto-reactive T lymphocytes play a pivotal role in autoimmune diseases such as MS.**

25. Mycosis fungoides is the principal form of cutaneous T-cell lymphoma. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010 12:320-327; DOI: 10.2353/jmoldx.2010.090123; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Mycosis fungoides accounts for over 75% of all primary cutaneous lymphomas. **This statement is NOT TRUE. Mycosis fungoides accounts for nearly 50% of all primary cutaneous lymphomas.**
b. It is difficult to distinguish, both clinically and histopathologically, mycosis fungoides from some reactive inflammatory dermatoses. **This statement is TRUE. Differentiation is especially difficult during early stages of the disease.**
c. The tumor cells of lymphomas harbor identically (clonally) rearranged T-cell receptor genes. **This statement is TRUE. Reactive skin disorders consist of cells with polyclonal T-cell receptor genes.**
d. T-cell receptor clonality testing is commonly performed on cases of suspected mycosis fungoides. **This statement is TRUE. The clonality testing provides additional evidence for diagnosis.**
e. Multiplex PCR assays can be used to detect clonally rearranged genes. **This statement is TRUE. The BIOMED-2 study made inter-laboratory comparison of these testing platforms possible.**

26. T-cell receptor (TCR) clonality is most often performed on *TCRG.* Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010 12:320-327; DOI: 10.2353/jmoldx.2010.090123; the authors of the reference article did not disclose any relevant financial relationships.]

a. There is a restricted repertoire of the V segments of the *TCRG* locus. **This statement is TRUE. There is also a restricted repertoire of the J segments of the *TCRG* locus.**
b. *TCRG* rearrangement occurs in both $\alpha\beta$ and $\gamma\delta$ T cells. **This statement is TRUE. This is due to the chronological order of TCR rearrangement.**
c. The BIOMED-2 study recorded *TCRG* rearrangement in 50% of T-cell malignancies. **This statement is NOT TRUE. The BIOMED-2 study recorded *TCRG* rearrangement in 89% of T-cell malignancies.**
d. Comparison of *TCRG* PCR results at several skin sites from the same patient may improve the accuracy of clonality testing. **This statement is TRUE. Serial analysis of skin biopsies over the course of disease may also improve accuracy.**
e. Clonality testing may be improved by examining both *TCRG* and *TCRB* rearrangements. **This statement is TRUE. In the algorithm proposed by Zhang et al, which integrates clinical information into molecular testing, the combined use of *TCRG* and *TCRB* clonality tests can maximize negative predictive value when clinical suspicion is moderately high and can maximize positive predictive value when the pretest probability is moderately low.**

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ANSWERS for CME Questions # 27-34
27c, 28b, 29e, 30d, 31d, 32b, 33c, 34d

27. Uveal melanoma is an intraocular tumor that often results in blindness or death. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:391-393; DOI: 10.2353/jmoldx.2010.100059; the author did not disclose any relevant financial relationship; and J Mol Diagn 2010, 12:461-468; DOI: 10.2353/jmoldx.2010.090220; J. William Harbour and Washington University may receive income based on a license of related technology by the University to Castle Biosciences, Inc.; this work was not supported by Castle Biosciences, Inc.; the other authors are employees of Washington University and did not disclose any other relevant financial relationships.]

a. Ophthalmologists have difficulty visually differentiating intraocular tumors from other eye lesions. **This statement is TRUE. These other lesions include choroidal hemangiomas, hemorrhages, or metastases to the eye.**
b. Historically, most patients with intraocular melanocyte tumors were treated by removal of the affected eye. **This statement is TRUE. To this day, there is no effective treatment for metastatic uveal melanoma.**
c. Patients whose affected eyes were removed had a lower chance of survival than patients who were treated by radiation. **This statement is NOT TRUE. There was no significant difference in survival between patients with affected eyes removed and patients with medium-sized tumors treated by radiation. Therefore, current treatment most often does not involve tissue removal.**
d. Nevi, melanocytoma (magnocellular nevus), and melanomas are three melanocytic lesions described in the uvea. **This statement is TRUE. There are no uveal counterparts to Spitz nevi, pigmented spindle cell tumors, and other benign melanocytic lesions that are described in the skin.**
e. In preparation for new treatment modalities, ophthalmologists are attempting to stratify patients in treatment groups. **This statement is TRUE. Therefore, accurate patient stratification by prognosis is critical.**

28. Despite clear phenotypic correlates, uveal melanoma prognosis stratification has been limited by technical concerns. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:391-393; DOI: 10.2353/jmoldx.2010.100059; the author did not disclose any relevant financial relationship; and J Mol Diagn 2010, 12:461-468; DOI: 10.2353/jmoldx.2010.090220; J. William Harbour and Washington University may receive income based on a license of related technology by the University to Castle Biosciences, Inc.; this work was not supported by Castle Biosciences, Inc.; the other authors are employees of Washington University and did not disclose any other relevant financial relationships.]

a. Morphology of uveal melanoma cells is associated with outcome. **This statement is TRUE. The Callender classification, which is based on the morphology of uveal melanoma cells, is the leading prognostic indicator for survival.**
b. Fine needle aspiration biopsies cannot confirm the diagnosis of uveal melanoma. **This statement is NOT TRUE. Sampling size is sufficient to confirm diagnosis, but not prognosis, of uveal melanoma.**
c. Fine needle aspiration biopsies do not provide good prognostic sampling of the tumor for Callender classification. **This statement is TRUE. While fine needle aspiration is sufficient for molecular diagnosis of uveal melanoma, the cells that confer the most advanced prognosis by morphological assessment are not necessarily captured by random sampling of the tumor.**

d. A cytogenetic profile has been strongly associated with tumor outcome. **This statement is TRUE. Many ophthalmic oncologists have attempted to use fine-needle aspiration biopsy to extract cells for cytogenetic analyses.**
e. Tumor heterogeneity has raised doubts about the sensitivity and specificity of cytogenetic profiling for establishing prognosis in uveal melanoma. **This statement is TRUE. Investigators have reported a heterogeneous distribution of cytogenetic markers of interest in uveal melanoma.**

29. Uveal melanoma is an aggressive cancer, and metastasis to the liver is often fatal. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:391-393; DOI: 10.2353/jmoldx.2010.100059; the author did not disclose any relevant financial relationship; and J Mol Diagn 2010, 12:461-468; DOI: 10.2353/jmoldx.2010.090220; J. William Harbour and Washington University may receive income based on a license of related technology by the University to Castle Biosciences, Inc.; this work was not supported by Castle Biosciences, Inc.; the other authors are employees of Washington University and did not disclose any other relevant financial relationships.]

a. Uveal melanoma often forms undetectable micrometastases before detection of the primary tumor. **This statement is TRUE. These micrometastases often form fatal metastatic tumors that are resistant to therapy.**
b. Early detection of uveal melanoma is key to successful therapy. **This statement is TRUE. Detection prior to metastasis or at early stages of metastasis may lead to more successful therapies.**
c. Gene expression profiling can differentiate uveal melanomas of low and high metastatic risk. **This statement is TRUE. This gene expression profile could be performed using a small number of discriminating genes on archival specimens and fine needle biopsy samples.**
d. Development of a practical, clinically feasible platform for analyzing the gene expression profile could benefit high-risk patients through intensified metastatic surveillance. **This statement is TRUE. This platform could also lead to earlier intervention for metastasis and stratification for entry into clinical trials of adjuvant therapy.**
e. In this study, Onken et al describe a new high-density hybridization-based microarray platform to test the gene expression profile of uveal melanoma patients. **This statement is NOT TRUE. In this study, Onken et al describe a PCR-based 15-gene assay. Tumor heterogeneity is thought not to be a factor in this test, although follow-up studies need to further address this issue.**

30. DNA ligase-based assays have been widely used to study genomic alterations. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:402-408; DOI: 10.2353/jmoldx.2010.090152; none of the authors disclosed any relevant financial relationships.]

a. DNA ligase-based assays covalently join two oligonucleotide probes adjacently on a DNA or RNA template. **This statement is TRUE. The reaction is efficient only when the two probes lie immediately adjacent to one another.**
b. DNA ligase-based assays were first designed to limit nonspecific probe binding. **This statement is TRUE. These assays were first described in 1984.**
c. DNA ligase-based assays have been developed for detection of disease-causing mutations and single-nucleotide polymorphisms. **This statement is TRUE. They are also used to detect sequence copy number variations and aberrant DNA methylation.**
d. Ligation of two probes by DNA ligase requires that the 5' probe be phosphorylated at its 5' end but the 3' probe can be dephosphorylated. **This statement is NOT TRUE. The 3' probe must be phosphorylated at is 5' end. The reaction is efficient only when there is a perfect match between template and probes at the ligation junction.**
e. DNA ligases are particularly sensitive to 3' mismatches. **This statement is TRUE. This sensitivity is used for allele discrimination in the oligonucleotide ligation assay (OLA), the ligase chain reaction (LCR), and for detection of DNA methylation using bisulfite-treated DNA as a template.**

31. Multiplex ligation-dependent probe amplification (MLPA) allows simultaneous screening of multiple target sequences. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:402-408; DOI: 10.2353/jmoldx.2010.090152; none of the authors disclosed any relevant financial relationships.]

a. MLPA was first developed as a technique for determining relative sequence copy numbers. **This statement is TRUE. This process uses probe pairs for up to 40 individual loci in a single reaction.**
b. Each probe used in MLPA consists of a target-specific sequence and a non-hybridizing tail. **This statement is TRUE. The non-hybridizing tails contains a universal primer binding site.**
c. At least one of the probes in each pair contains a "stuffer sequence" of defined length between the primer binding site and the specific target sequence. **This statement is TRUE. This "stuffer sequence" is a means to modulate the length of the ligation product.**

d. The first step in MLPA is the ligation of the probe pairs, followed by hybridization to the template and amplification of the ligated probes with a common primer pair. **This statement is NOT TRUE. The probe pairs are first hybridized to the template and then joined by ligation. Next, the ligated products are amplified with a common primer pair. The probe-derived amplicons can then be separated by electrophoresis according to size.**
e. A major drawback of MLPA lies in the requirement for long probes, which cannot be made efficiently by chemical synthesis. **This statement is TRUE. Serizawa et al describe an alternative and simple approach to MLPA analysis, which involves the use of multiple short synthetic probes for each target site. The length of the probe-derived amplicons can be preset by the number and lengths of probes in the individual probe sets.**

32. Rearrangements in the mixed lineage leukemia *(MLL)* gene are associated with the development of diverse leukemias. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:441-452; DOI: 10.2353/jmoldx.2010.090214; none of the authors disclosed any relevant financial relationships.]

a. *MLL* rearrangements are found in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). **This statement is TRUE. These rearrangements are also found in mixed phenotype leukemia and acute leukemias associated with topoisomerase II-based chemotherapy.**
b. Overall, rearrangements of *MLL* occur in approximately 25% of acute leukemia cases in adults and children. **This statement is NOT TRUE. Rearrangements of *MLL* occur in approximately 6% of acute leukemia cases in adults and children.**
c. *MLL* rearrangements account for 80% of cases of ALL in children under 12 months of age. **This statement is TRUE. *MLL* rearrangements are significantly more frequent in specific patient cohorts than in the overall leukemia population.**
d. In AML, rearrangements of *MLL* are typically associated with a myelomonoblastic or monoblastic phenotype (M4 and M5 subtypes). **This statement is TRUE. These rearrangements are more frequent in children, representing 9% to 12% of pediatric cases and approximately 65% of infant cases.**
e. *MLL* rearrangement is frequent in mixed phenotype acute leukemia in children. **This statement is TRUE. Therapy-related AML, occurring in patients who have been previously treated with chemotherapeutic drugs that inhibit DNA topoisomerase II, also show a high frequency of rearrangements involving *MLL*.**

33. The *MLL* gene contains over 37 exons and spans 200 kilobases. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:441-452; DOI: 10.2353/jmoldx.2010.090214; none of the authors disclosed any relevant financial relationships.]

a. The majority of recurrent *MLL* translocation breakpoints occur within an 8.3 kb breakpoint cluster region (*BCR*). **This statement is TRUE. *BCR* is located between exons 8 and 14.**
b. More than 80 recurrent chromosomal translocations disrupting *MLL* have been identified. **This statement is TRUE. Moreover, greater than 50 *MLL* partner genes have been defined.**
c. Three translocations account for approximately 80% of all *MLL*-associated leukemia. **This statement is NOT TRUE. Five translocations account for approximately 80% of all *MLL*-associated leukemia. The five most frequent *MLL* rearrangements include t(4;11)(q21;q23), *AFF1(AF4)/MLL*; t(6;11)(q27;q23), *MLLT4(AF6)/MLL*; t(9;11)(p22;q23), *MLLT3(AF9)/MLL;* t(11;19)(q23;p13.1), *MLL/ELL*; and t(11;19)(q23;p13.3), *MLL/MLLT1(ENL)*.**
d. Patients with acute leukemia with *MLL* rearrangements generally have a less favorable prognosis and response to chemotherapy than patients without these rearrangements. **This statement is TRUE. Recent studies have suggested that the specific *MLL* rearrangement partner may also influence response to therapy and overall prognosis, depending on the clinical context.**
e. Conventional cytogenetics may fail to detect nearly one third of *MLL* rearrangements. **This statement is TRUE. Fluorescence *in situ* hybridization (FISH) has emerged as the modality of choice for detection of such rearrangements.**

34. Shiga toxin (ST)-producing *Escherichia coli* (STEC) are important causative agents of enterocolitis food poisoning. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:469-475; DOI: 10.2353/jmoldx.2010.090221; none of the authors disclosed any relevant financial relationships.]

a. STEC can lead to a wide array of clinical manifestations ranging from mild presentation of acute gastritis to bloody diarrhea. **This statement is TRUE. STEC can occasionally lead to complications such as hemolytic uremic syndrome as well.**
b. STEC infection is most severe in pediatric and elderly patients. **This statement is TRUE. Adults do not present with severe symptoms in most cases.**
c. The O157:H7 serotype of STEC is considered a major public health concern in the food production industry. **This statement is TRUE. Several studies suggest that up to 50% of STEC illness is caused by serotypes other than O157, of which there are over 100.**

d. O157 STEC are routinely screened for in the clinical microbiology laboratory by selective plating on mannitol salt agar, which exploits the inability of most O157 strains to ferment mannitol. **This statement is NOT TRUE. O157 STEC are routinely screened for in the clinical microbiology laboratory by selective plating on sorbitol MacConkey media (SMAC), which exploits the non-sorbitol-fermenting phenotype of most O157 strains.**

e. The Centers for Disease Control and Prevention guidelines for the detection of STEC in relation to acute community-acquired diarrhea recommend specific testing for Shiga toxins or their genetic determinants in addition to traditional culture. **This statement is TRUE. Chui et al compare conventional and real-time PCR assays targeting the *stx1* and *stx2* Shiga toxin coding sequences to identify STEC in enriched stool samples.**

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ANSWERS for CME Questions # 35-42
35e, 36b, 37d, 38a, 39c, 40a, 41b, 42e

35. Next generation sequencing (NGS) refers to high-throughput sequencing technologies in which clonally amplified DNA templates or single DNA molecules are sequenced in a massively parallel fashion in a flow cell. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:539-551; DOI: 10.2353/ jmoldx.2010.100043; the authors of the referenced article did not disclose any relevant financial relationships.]

a. NGS can be conducted via a stepwise iterative process. **This statement is TRUE. Alternately, NGS can be conducted in a continuous real-time manner.**
b. In NGS, each clonal template or single molecule is "individually" sequenced. **This statement is TRUE. Each individual sequence can be counted among the total sequences generated.**
c. NGS provides a high-throughput combination of qualitative and quantitative sequence information. **This statement is TRUE. These data have allowed analyses that were previously either not technically possible or cost prohibitive.**
d. NGS is the method of choice for large scale complex genetic analyses including whole genome and transcriptome sequencing and metagenomic characterization of microbial species in environmental and clinical samples. **This statement is TRUE. NGS is also used to elucidate DNA binding sites for chromatin and regulatory proteins and for targeted resequencing of regions of the human genome identified by linkage analyses and genome wide association studies.**
e. NGS has experienced wide dissemination throughout molecular diagnostics because implementation in the clinical laboratory has not required expertise in molecular biology techniques. **This statement is NOT TRUE. NGS has experienced wide dissemination throughout biomedical research; however, its translation into molecular diagnostics is just beginning and will require expertise in both molecular biology techniques and bioinformatics.**

36. A major first step in NGS is preparation of a "library" comprised of DNA fragments ligated to platform-specific oliognucleotide adapters. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:539-551; DOI: 10.2353/ jmoldx.2010.100043; the authors of the referenced article did not disclose any relevant financial relationships.]

a. The input nucleic acid for library construction can be genomic DNA or standard or long-range PCR amplicons. **This statement is TRUE. The input nucleic acid can also be cDNA.**
b. The goal of library construction is to generate random overlapping fragments typically in the size range of 1,000-6,000 base pairs depending on platform and application requirements. **This statement is NOT TRUE. The goal of library construction is to generate random overlapping fragments typically in the size range of 150-600 base pairs depending on platform and application requirements.**
c. Fragmentation by nebulization uses compressed air flowing through an aqueous nucleic acid solution for several minutes. **This statement is TRUE. This approach is prone to volume loss and potential sample cross-contamination.**

d. Fragmented nucleic acids have terminal overhangs that require blunt end repair and phosphorylation. **This statement is TRUE. Commonly, fragments are incubated with Klenow (3' to 5' exonuclease minus), T4 DNA polymerase (3' to 5' exonuclease plus), and polynucleotide kinase in the presence of dNTPs and ATP.**
e. Ligation products are often size-separated by gel electrophoresis, and a specific size range is selected that is compatible with a given platform or application. **This statement is TRUE. The adapter-modified fragments constitute the "library" of overlapping sequences.**

37. NGS chemistries include pyrosequencing, sequencing by reversible dye terminators, and sequencing by sequential ligation of oligonucleotide probes. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:539-551; DOI: 10.2353/ jmoldx.2010.100043; the authors of the referenced article did not disclose any relevant financial relationships.]

a. For pyrosequencing, dATP, dCTP, dGTP, or dTTP and polymerase are sequentially flowed over a picotitre plate containing bead-bound, clonally-amplified DNA templates. **This statement is TRUE. When incorporation of a complementary nucleotide occurs on a growing strand in an individual well, pyrophosphate is released, which drives luciferase-mediated light generation in the well.**
b. Luminescence intensity in pyrosequencing is directly proportional to the number of nucleotides incorporated. **This statement is TRUE. Therefore, homopolymer signals are more intense than single base additions and are length-dependent.**
c. Sequencing by reversible dye terminators incorporates a mixture of four fluorescently unique dye terminators that are simultaneously introduced into the flow cell along with DNA polymerase. **This statement is TRUE. Incorporation of complementary bases into individual clusters is recorded by virtue of base-specific fluorescent emission spectra. The fluor and termination moieties, linked to the nucleotide base and 3' deoxyribose sugar position, respectively, are then cleaved and washed away. Successive cycles of dye terminator mixture and DNA polymerase introduction, incorporation, and cleavage yield chain elongation.**
d. Sequencing by ligation involves the simultaneous introduction of either fluorescently duplicated oligonucleotide probes containing specific interrogation nucleotides or degenerate nucleotides. **This statement is NOT TRUE. Sequencing by ligation involves the iterative introduction of combinations of fluorescently unique oligonucleotide probes containing specific interrogation nucleotides and degenerate nucleotides. After annealing and ligation, fluorescence is recorded, and the labeled section of each probe is cleaved and washed away.**
e. Resulting read lengths vary between chemistries and have been progressively increasing as each technology evolves. **This statement is TRUE. Of the first wave technologies, pyrosequencing read lengths are the longest at greater than 400 bases.**

38. Each NGS platform generates different read lengths that range from short reads (e.g, 35 bases) to reads greater than 500 bases. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:539-551; DOI: 10.2353/ jmoldx.2010.100043; the authors of the referenced article did not disclose any relevant financial relationships.]

a. For a number of applications, including targeted resequencing, CHiP-Seq, and RNA-Seq, longer reads are required to be highly informative and adequate. **This statement is NOT TRUE. Short reads are highly informative and adequate for a number of applications, including targeted resequencing, CHiP-Seq, and RNA-Seq.**
b. The advantage of longer reads is evident in applications of *de novo* genome assembly and when sequencing through areas of repetitive DNA and targets that share regions of high homology. **This statement is TRUE. Some examples of targets that share regions of high homology are members of a related gene family, including functional genes and pseudogene analogs.**
c. Most platforms have the option of being able to sequence both ends of library fragments, termed pair-end or mate-pair sequencing. **This statement is TRUE. Pair-end sequencing effectively doubles the amount of sequence obtained, facilitates alignment, and improves detection of insertions and deletions occurring between the pair ends.**
d. With respect to accuracy, NGS chemistries are prone to errors occurring in individual reads at frequencies in the 0.5 to 2% range, depending on the platform used. **This statement is TRUE. Errors are primarily substitutions and secondarily single base insertions and deletions (with erroneous indels being more pronounced in homopolymer tracts). Therefore, nucleotide variant changes (e.g., from a reference sequence) cannot be accurately relied upon if present in only a single read.**
e. The number of times a nucleotide base has been sequenced is referred to as its "coverage." **This statement is TRUE. It is not fully defined how much coverage is needed for accurate sequencing, if accuracy is defined as ~ 99.5 to 99.9 %. This varies with platform but empirical results from the literature have suggested as few as 4 to 5 reads per allele, whereas most groups require 10 to 30 reads per allele.**

39. Caveolin is an oligomerized protein that coats caveolae, invaginations of the plasma membrane. Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:562-565; DOI: 10.2353/jmoldx.2010.100093 and J Mol Diagn 2010, 12:712-717; DOI: 10.2353/jmoldx.2010.090180; the authors of the referenced articles did not disclose any relevant financial relationships.]

a. Caveolin-1 is expressed in cell of various tissues, including the mesenchyme, endothelium, neuronal tissues, and some epithelial cells. **This statement is TRUE. Caveolin-1 is co-expressed with caveolin-2 in these tissues.**
b. The *caveolin-1* (*CAV1*) gene can be translated as either a full-length protein, the α-isoform, or as a protein that lacks the first 32 amino acids, the β-isoform. **This statement is TRUE. The *CAV1* gene is composed of 3 exons.**
c. Caveolae are primarily involved in phagocytosis and lysosomal degradation. **This statement is NOT TRUE. Caveolae are involved in various cellular processes, including cholesterol homeostasis, vesicular transport, cell migration, cell cycle, and cell polarity.**
d. Caveolin-1 directly interacts with pro-proliferative molecules, including EGFR, ERBB2, and PI3K, through the caveolin-scaffolding domain. **This statement is TRUE. Caveolin-1 negatively controls signaling pathways regulating cell proliferation, differentiation, apoptosis, adhesion, and invasion.**
e. The transcript and protein levels of *CAV1* are down-regulated in cancer cell lines. **This statement is TRUE. Reduced levels of this protein in NIH3T3 cells leads to oncogenic transformation.**

40. A sporadic mutation in the *CAV1* gene leads to a proline-to-leucine substitution at position 132 (P132L). Based on the referenced article and related Commentary, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:562-565; DOI: 10.2353/jmoldx.2010.100093 and J Mol Diagn 2010, 12:712-717; DOI: 10.2353/jmoldx.2010.090180; the authors of the referenced articles did not disclose any relevant financial relationships.]

a. The P132L mutation has an activating effect through the dimerization of caveolin-1 within the caveolae. **This statement is NOT TRUE. The P132L mutation has a dominant negative effect through the misfolding of caveolin-1 within the Golgi complex.**
b. Overexpression of caveolin-1 is common in various cancer types. **This statement is TRUE. These data contradict the suggested tumor suppressor function of caveolin-1.**
c. The initial report on the P132L mutation stated that 15 out of 92 primary breast tumors harbored this mutation. **This statement is TRUE. However, a later study reported that the P132L mutation was not detected in any of 55 breast cancer specimens, using the same method as described in the initial article.**
d. A subsequent report on the P132L mutation determined that it was specific to estrogen receptor (ER)-positive breast cancers. **This statement is TRUE. The mutation was found in 6 out of 32 ER-positive tumors (35%) but in none of the 23 ER-negative tumors.**
e. Koike et al detected no P132L caveolin-1 mutations in a panel of breast and other cancers. **This statement is TRUE. Koike et al used standard direct sequencing as well as a more sensitive method for paraffin sections that could detect the mutation allele at a rate of 0.1% among wild-type allele copies.**

41. Transplant glomerulopathy (TG) is a disease of the kidney allograft initiated by endothelial injury. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:653-663; DOI: 10.2353/jmoldx2010.090101; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Morphologically, in TG there is widening of the subendothelial space with accumulation of debris, mesangial interpositioning, and matrix deposition in the glomerular capillary wall. **This statement is TRUE. There is also capillary wall double-contouring in the absence of immune complex deposition.**
b. In TG, light microscopy may show endothelial cell separation from the glomerular basement membrane prior to electron microscopic changes. **This statement is NOT TRUE. Electron microscopy may show endothelial cell separation from the glomerular basement membrane prior to light microscopic changes.**
c. Evidence of chronic injury, including interstitial fibrosis and tubular atrophy, may accompany a TG lesion. **This statement is TRUE. Interstitial fibrosis and tubular atrophy are the hallmarks of chronic allograft nephropathy.**
d. Clinical presentation of TG often occurs a year or more after transplantation. **This statement is TRUE. However, in the context of protocol kidney biopsies, light microscopic changes may be seen earlier, with associated proteinuria, hypertension, and a progressive decline in function culminating in graft loss.**
e. There is no specific effective therapeutic strategy for TG beyond augmentation of immunosuppression. **This statement is TRUE. Thus, identifying pathogenic mediators not only for therapeutic purposes but also for early identification may lead to improved outcomes.**

42. Molecular amplification assays potentially offer the sensitivity and speed needed to manage an influenza outbreak. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:664-669; DOI: 10.2353/ jmoldx.2010.090225; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Standardized RT-PCR assays specific for the 2009 novel influenza A/H1N1 strain of influenza were not initially available. **This statement is TRUE. This lack of availability left many laboratories to diagnose this infection through indirect means.**
b. The group of Kaul et al has implemented a rapid RT-PCR/melt-curve assay designed to differentiate seasonal influenza A subtypes H1N1 and H3N2. **This statement is TRUE. This approach was initially developed for viral subtyping to guide clinicians to the appropriate antiviral therapy.**
c. Rapid determination of influenza A subtype is essential for determining optimal therapy and for prudent use of antiviral agents. **This statement is TRUE. Antiviral resistance has risen during recent years, with the majority of seasonal H1N1 strains no longer sensitive to oseltamivir and seasonal H3N2 strains largely resistant to adamantanes.**
d. The design of the RT-PCR assay reported by Kaul et al exploits minor variations in a relatively conserved sequence within the matrix protein gene. **This statement is TRUE. Not surprisingly, the novel H1N1 strain of influenza that appeared in the spring of 2009 had a distinct melting temperature consistent with the published matrix gene sequence and the sequence of the fluorescence resonance energy transfer (FRET) probes used in an assay designed to differentiate seasonal influenza A subtypes H1N1 and H3N2.**
e. The assay designed by Kaul et al allowed definitive diagnosis of the 2009 influenza H1N1 from nasopharyngeal swabs within twenty minutes of specimen arrival in the clinical laboratory. **This statement is NOT TRUE. This assay allowed definitive diagnosis of the 2009 influenza H1N1 from nasopharyngeal swabs within hours after arrival in the clinical laboratory.**

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

ANSWERS for CME Questions # 43-50
43e, 44b, 45c, 46a, 47d, 48d, 49a, 50d

43. Patient samples for pathology analysis can be collected either surgically or cytologically. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12: 739-745; DOI: 10.2353/jmoldx.2010.090238; the authors of the referenced manuscript did not disclose any relevant financial relationships.]

a. Surgical samples can be collected by either core needle or surgical biopsy. **This statement is TRUE. These samples are typically processed as formalin-fixed, paraffin-embedded (FFPE) samples.**
b. FFPE surgical pathology specimens currently comprise the foundation for retrospective clinical genetic profiling studies. **This statement is TRUE. FFPE samples are also typically required for prospective patient enrollment in clinical trials.**
c. Fine-needle aspiration cytology (FNAC) procedures may be used for rapid, cost-effective, and accurate diagnosis. **This statement is TRUE. The rapidity of FNAC procedures may result in reduced patient morbidity.**
d. Body fluids and epithelial scrapes are another source of cytology preparation. **This statement is TRUE. These sources include pleural effusion, ascetic fluid, cerebral-spinal fluid (CSF), cervical Pap smears, and bronchial and esophageal brushings.**
e. Cytologic preparations represent an estimated 80% to 90% of archival hospital pathology specimens. **This statement is NOT TRUE. Cytologic preparations represent an estimated 10% to 20% of archival hospital pathology specimens and may be significantly enriched for particular pathologic disorders.**

44. Higher-resolution genotyping arrays are now used for tissue molecular profiling. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12: 739-745; DOI: 10.2353/jmoldx.2010.090238; the authors of the referenced manuscript did not disclose any relevant financial relationships.]

a. Unbiased whole genome amplification requires higher molecular weight DNA than is often available from FFPE samples. **This statement is TRUE. High molecular weight DNA is often compromised in FFPE samples over time.**
b. FFPE samples more than a few years old are preferable for array-based genome profiling studies. **This statement is NOT TRUE. Array-based genome profiling studies may be data limited when analyzing archived FFPE samples more than a few years old.**
c. Needle aspirate and fluid samples have been largely unutilized for molecular profiling. **This statement is TRUE. They are unutilized despite their presence in hospital archives alongside FFPE samples.**
d. Comparative genomic hybridization could be used to support a primary cytologic diagnosis of malignancy in the clinical setting. **This statement is TRUE. Little information is available regarding practical details and prospects for genomic scale molecular profiling of routinely processed archival and prospective cytology specimens.**
e. Successful PCR on archival cytology samples up to 5 years old has been reported. **This statement is TRUE. These studies did not assess the DNA yields and integrities from various kinds of cytologic preparations.**

45. Cytopathology specimens are potentially excellent sources of patient materials for clinical molecular profiling. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12: 739-745; DOI: 10.2353/jmoldx.2010.090238; the authors of the referenced manuscript did not disclose any relevant financial relationships.]

a. Cytopathology specimens can be used for retrospective genomic analyses and prospective sample collection for individualized therapy. **This statement is TRUE. They can also be used for eligibility review for clinical trial enrollment.**
b. Fine-needle aspiration cytopathology (FNAC) samples up to 16 years old yield high quantities of genomic DNA. **This statement is TRUE. This DNA is suitable for high-resolution genomic and epigenomic profiling.**
c. Matched Papanicalaou and Diff-Quik FNAC samples exhibited identical patterns of DNA preservation and DNA integrity. **This statement is NOT TRUE. Diff-Quik versus Papanicalaou cytologic staining processes show remarkable differences in DNA preservation, possibly implicating hematoxylin as a DNA-damaging agent.**
d. Over 95% of malignant aspirates in the study by Killian et al manifested clearly discernible genomic aberrations. **This statement is TRUE. This suggests that diagnostic FNAC samples are often inherently of sufficient purity for genomic scale molecular profiling.**
e. FNAC samples were suitable for aberration detection on high-resolution comparative genomic hybridization array. **This statement is TRUE. They were also suitable for examining DNA methylation and single nucleotide polymorphism genotyping platforms.**

46. Lynch syndrome, or hereditary nonpolyposis colorectal cancer (CRC), is a common cancer predisposition syndrome. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12: 757-764; DOI: 10.2353/jmoldx.2010.090240; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Lynch syndrome accounts for about 20% of all CRC cases. **This statement is NOT TRUE. Lynch syndrome accounts for about 2% of all CRC cases.**
b. Patients affected with Lynch syndrome have about an 80% lifetime risk of developing CRC. **This statement is TRUE. Germline mutations predispose patients to Lynch syndrome.**
c. Malignancies of the endometrium, skin, and bladder are associated with this syndrome. **This statement is TRUE. Lynch syndrome is also associated with malignancies of the ovaries and kidney.**
d. Lynch syndrome is an autosomal dominant condition. **This statement is TRUE. An affected individual needs to have only one copy of the affected allele.**
e. The discovery of a predisposing mutation in Lynch syndrome families and the concomitant increase in surveillance have been shown to significantly reduce mortality. **This statement is TRUE. Therefore, it is of paramount importance to classify the pathogenicity associated with unclassified variants to determine their role in cancer predisposition.**

47. Lynch syndrome is characterized by mutations in mismatch repair (MMR) genes. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12: 757-764; DOI: 10.2353/jmoldx.2010.090240; the authors of the referenced article did not disclose any relevant financial relationships.]

a. The MMR system is a postreplicative DNA repair system that recognizes and repairs base-base mismatches and insertion/deletion loops that occur during DNA replication. **This statement is TRUE. These mutations also must escape the proofreading activity of the DNA polymerases.**
b. MMR proteins have been implicated in signaling DNA damage. **This statement is TRUE. This signaling results in cellular apoptosis.**
c. Germline mutations of the MMR genes *MLH1, MSH2,* and *PMS2* have been implicated in Lynch syndrome. **This statement is TRUE. *PMS1* and *MSH6* have also been implicated in Lynch syndrome.**
d. Ninety percent of all known mutations are found in the *MLH1* and *PMS2* genes. **This statement is NOT TRUE. Ninety percent of all known mutations are found in the *MLH1* and *MSH2* genes.**
e. Truncating mutations are the most common type of mutation identified in the *MLH1* and the *MSH2* genes. **This statement is TRUE. Truncating mutations lead to transcripts with premature termination codons that are generally degraded by nonsense-mediated RNA decay, which functions to eliminate truncated proteins that may have deleterious effects.**

48. Tumors with defective MMR function are characterized by expansion/contraction of microsatellite regions. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12: 757-764; DOI: 10.2353/jmoldx.2010.090240; the authors of the referenced article did not disclose any relevant financial relationships.]

a. Tumors with defective MMR function exhibit microsatellite instability (MSI). **This statement is TRUE. MSI is seen in about 85% of Lynch syndrome tumors.**
b. Individuals diagnosed with cancer at a younger than average age are generally referred to predictive genetic testing programs. **This statement is TRUE. Individuals with familial clustering of colorectal or other Lynch syndrome-associated cancers are also generally referred to predictive genetic testing programs.**
c. MSI testing is carried out on tumor DNA. **This statement is TRUE. Tumor DNA acts as a surrogate marker of defective MMR function. Although MSI testing is a robust assay of MMR activity, it is not a universal indicator of pathogenicity since it is possible for mutations to affect other functions of the MMR pathway.**
d. Indication of protein deficiency by immunohistochemistry (IHC) definitively links the genetic alteration to the loss of protein expression. **This statement is NOT TRUE. Indication of protein deficiency by IHC does not definitely link the genetic alteration to the loss of protein expression. Some tumors show equivocal staining patterns with intermediate levels of expression, which cannot be clearly distinguished by IHC.**
e. Although MSI testing is a robust assay of MMR activity, it is not a universal indicator of pathogenicity in Lynch syndrome. **This statement is TRUE. It is possible for mutations to affect other functions of the MMR pathway.**

49. Conventional karyotyping has been the primary technique of prenatal diagnosis since the 1970s. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:828-834; DOI: 10.2353/jmoldx.090224; the authors of the referenced article did not disclose any relevant financial relationships.]

a. The reporting time for the majority of karyotyping tests is around 2 to 3 days. **This statement is NOT TRUE. The reporting time for the majority of karyotyping tests is around 2 to 3 weeks.**
b. Automated methods are now being introduced to reduce the turnaround time of prenatal diagnoses. **This statement is TRUE. The automated methods also decrease the workload of technicians performing the tests.**
c. Three rapid aneuploidy tests are used to detect the most common aneuploidies. **This statement is TRUE. The most common aneuploidies are trisomies 13, 18, 21, and the sex chromosomes.**
d. Fluorescence *in situ* hybridization (FISH), quantitative fluorescence PCR (QF-PCR), and multiplex ligation-dependent probe amplification (MLPA) are methods that can reduce the reporting time compared to karyotyping. **This statement is TRUE. These methods reduce the reporting time to 24 to 48 hours, allowing earlier decision making for pregnancy management.**
e. The false-negative rate is low with rapid aneuploidy tests. **This statement is TRUE. FISH, QF-PCR, and MLPA have all been validated for prenatal diagnosis.**

50. Array-based comparative genomic hybridization (aCGH) has higher resolution than the currently used rapid aneuploidy tests. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2010, 12:828-834; DOI: 10.2353/jmoldx.090224; the authors of the referenced article did not disclose any relevant financial relationships.]

a. aCGH provides information about the DNA copy number of the whole genome. **This statement is TRUE. This information is not provided by all of the rapid aneuploidy tests.**
b. aCGH has an average reporting time of 6 to 16 days. **This statement is TRUE. The reporting time is dependent on whether fresh or cultured media is used.**
c. aCGH is less labor-intensive than karyotyping. **This statement is TRUE. aCGH is rapid and is suitable for automation.**
d. aCGH is cheaper than traditional rapid aneuploidy tests or karyotyping. **This statement is NOT TRUE. Cost is a detriment to acceptance of aCGH testing; however, reductions in cost may lead to replacement of karyotyping in the future.**
e. Unlike aCGH, QF-PCR can be used alone in a large number of samples studied in the prenatal laboratory. **This statement is TRUE. Adoption of this practice would reduce both the workload in cytogenetic laboratories and the anxiety of parents awaiting results.**