A Technical Advance article on noncontinuously binding loop-out primers and three research articles on ultrasensitive human DNA detection, epidermal growth factor receptor (EGFR) mutation detection in cerebrospinal fluid, and molecular diagnosis of invasive aspergillosis were selected for the September 2014 JMD CME Program in Molecular Diagnostics. The authors of the referenced articles, the planning committee members, and staff have no relevant financial relationships with commercial interests to disclose.


Upon completion of this month’s journal-based CME activity, you will be able to:

- Understand that genetic polymorphisms are a potential source of genotyping errors.
- Describe noncontinuously binding (loop-out) oligonucleotide hybridization probes.
- Explain the advantages of non-myeloablative conditioning regimens.
- Define and understand short tandem repeats (STRs) and STR analysis.
- Describe single nucleotide polymorphisms (SNPs).
- Understand that brain metastases are a frequent complication of non-small cell lung cancer (NSCLC).
- Describe the importance of identifying biomarkers in cerebrospinal fluid (CSF) in patients with brain metastases.
- Understand epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI).
- Define invasive aspergillosis (IA).
- Describe galactomannan (GM) and how it is measured.
- Explain nucleic acid sequenced-based amplification (NASBA).
1. Genetic polymorphisms are a potential source of genotyping errors. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:477-480.]

- a. Sequence variants that occur in PCR primer binding sites can cause allele-specific PCR dropout, leading to false-negative or apparent homozygous results.
- b. A polymorphism in intron 2 of MEN1, c.-23-16C>G (rs509606), has an allele frequency of approximately 35% in white Europeans.
- c. Although rs509606 lies outside the PCR primer binding sites, the allele containing this variant preferentially amplifies in heterozygous individuals, resulting in dropout of the wild-type allele.
- d. Amplification bias in favor of rs509606 has been reported to occur because rs509606 changes the stability of G-quadruplex- and i-motif-like DNA secondary structures in the amplicon.

2. Noncontinuously binding (loop-out) oligonucleotide hybridization probes have been described for molecular haplotyping and multiplex genotyping of nonadjacent sequence variants. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:477-480.]

- a. In loop-out probes, stretches of nucleotides are omitted between two or more nearby regions to be tested.
- b. Flanking segments of eight nucleotides on either side of the omitted sequence are sufficient for loop-out probes to reliably bind to multiple regions of interest.
- c. Additional PCR and sequencing reactions increase the overall costs of running a test.
- d. Using a modified PCR or additives for a single reaction is disruptive to the clinical laboratory workflow.

3. Myeloablative conditioning and allogeneic stem cell transplantation (alloSCT) have historically been limited to the treatment of lethal hematologic malignancies in children or young adults. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:495-503.]

- a. The advent of highly immunosuppressive, non-myeloablative regimens has expanded the clinical use of alloSCT to include middle-aged, fit patients with hematologic malignancies.
- b. AlloSCT is used for patients with non-malignant disorders, such as sickle cell disease (SCD).
- c. Non-myeloablative conditioning regimens offer the additional safeguard of recovery of autologous hematopoiesis in the event of graft rejection.
- d. Non-myeloablative conditioning regimens may be a safer option in patients at risk for immune-mediated rejection of the donor graft.

4. Chimerism testing at set intervals is an effective method for detecting graft rejection or recurrence of the original hematopoietic neoplasm after allogeneic hematopoietic stem cell transplantation (HSCT). Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:495-503.]

- a. Decades ago, bone marrow engraftment monitoring was performed using Southern blotting and minisatellite or variable number of tandem repeats loci.
- b. Today, short tandem repeat (STR) loci are most commonly used for monitoring bone marrow engraftment.
- c. Each STR unit is 1 to 3 bases in length.
- d. STRs are composed of 10 to 60 tandemly repeated units.

5. Human identity testing is critical to the fields of forensics, paternity, and hematopoietic stem cell transplantation. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:495-503.]

- a. STRs are widely distributed throughout the human genome.
- b. STRs are highly variable between individuals, and therefore allow for excellent differentiation between individuals, including patient and donor, even if they are closely related.
- c. Most laboratories use multiplex PCR based kits, originally developed for forensics analysis using Combined DNA Index System (CODIS) loci.
- d. STR analysis always involves PCR amplification using fluorescently labeled primers followed by amplicon separation by polyacrylamide gel electrophoresis.

6. STRs and single nucleotide polymorphisms (SNPs) can be used to monitor bone marrow engraftment. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:495-503.]

- a. SNPs are theoretically superior to STR-based analysis because analysis of STR loci by capillary electrophoresis is relatively insensitive (limit of detection 5 to 10%).
- b. Microsatellite alleles of varying length amplify with different efficiencies, thus making them inherently biased.
- c. STR amplification can be difficult in the setting of highly degraded DNA.
- d. SNPs are less attractive as targets due to their inherently lower informativity, requiring many more SNPs to be tested to identify those that distinguish donor from recipient.
7. Brain metastases are a frequent complication of non-small cell lung cancer (NSCLC), especially in patients with lung adenocarcinoma. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:558-563.]

   a. Brain metastases are observed in 50% to 75% of patients at initial diagnosis of NSCLC.
   b. Patients with metastases are unable to undergo surgical resection of primary or cranial metastatic tumors to provide specimens for histopathological or biomarker studies.
   c. Tumor-derived DNA can be secreted into body fluids surrounding the tumor.
   d. The development of methods to identify potential molecular biomarkers from nonsurgical biopsy samples, such as cerebrospinal fluid (CSF), may facilitate the identification of clinically relevant gene signatures in patients with metastatic brain tumors.

8. Brain metastases are diagnosed according to clinical presentation, primary malignant tumor, and radiological imaging. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:558-563.]

   a. If the computed tomography or magnetic resonance imaging (MRI) aspect is atypical, tissue diagnosis, including brain tumor or CSF cytology, is necessary.
   b. In certain clinical situations, MRI would not be helpful for patients with leptomeningeal metastases in which positive CSF cytology results are <70%.
   c. The detection of oncogenes in CSF might facilitate the diagnosis of brain metastases in patients with lung adenocarcinoma.
   d. The detection of epidermal growth factor receptor (EGFR) gene status in tumor-derived free DNA in CSF might be a good clinical option.

9. EGFR tyrosine kinase inhibitor (TKI) is a small-molecular agent capable of penetrating brain tissue. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:558-563.]

   a. EGFR-TKI has been found to significantly improve survival rates and tumor responses in lung adenocarcinoma patients with metastatic brain tumors that harbor EGFR-activating mutations.
   b. The most common target populations for treatment with EGFR-TKI are males with adenocarcinomas.
   c. EGFR gene status can only be detected in approximately 10% of patients with advanced NSCLC in China.
   d. The limited availability of testing technology and economic factors are the leading causes of the low detection rate of NSCLC in China.

10. Invasive aspergillosis (IA), an opportunistic fungal infection, has been increasingly recognized as a major cause of morbidity and mortality in immunocompromised patients. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:584-590.]

    a. IA remains difficult to diagnose despite great advances in imaging and antigen-based serological detection.
    b. Because early diagnosis is important for improved outcomes, efforts have been devoted to develop diagnostic assays targeting fungal biomarkers that offer the potential for new paradigms in prevention and early treatment of IA.
    c. Galactomannan (GM), a fungal biomarker, can be released into the serum and bronchoalveolar lavage (BAL) fluid during fungal infection.
    d. GM is a polysaccharide component of the fungal nuclear membrane.

11. Measurement of GM can be achieved by using a commercially available ELISA kit, which has been approved by the US Food and Drug Administration. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:584-590.]

    a. False-negative results with the GM ELISA assay kit may occur because of cross-reactivity with nutritional preparations.
    b. The GM ELISA assay lacks species-specificity and is unable to differentiate among Aspergillus spp.
    c. With the advent of PCR, it has become possible to detect pathogen genes in clinical samples, allowing early diagnosis of IA.
    d. Conventional PCR and real-time quantitative PCR (qPCR) amplification techniques lack standardization and clinical validation for IA.

12. Nucleic acid sequenced-based amplification (NASBA) is a RNA-directed isothermal transcription-based amplification process that specifically amplifies RNA even in the presence of genomic DNA. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:584-590.]

    a. The amplification efficiency of NASBA has been shown to be more robust than PCR.
    b. Amplification using NASBA yields >10^{10} amplicons in 45 minutes.
    c. The advantages of NASBA over PCR (simplicity, speed, and sensitivity) have stimulated interest in evaluating its ability to detect Aspergillus RNA in clinical samples.
    d. The authors measured circulating Aspergillus GM, DNA, and RNA in blood samples of 80 patients; the data support the great potential of NASBA and qPCR, singly or in combination, for diagnosis of IA in high-risk populations.