A Technical Advance article on genetic evaluation of stillbirth using formalin-fixed, paraffin-embedded (FFPE) tissue and research articles on the development of a genomic DNA reference material panel for myotonic dystrophy type 1 (DM1) genetic testing and the development of next-generation sequencing assays for mitochondrial and nuclear genes associated with mitochondrial disorders were selected for the July 2013 JMD CME Program in Molecular Diagnostics.

Questions #1-6 are based on: Rowe LR, Thaker HM, Opitz JM, Shiffman JD, Haddadin ZM, Erickson LK, Smith ST: Molecular inversion probe array for the genetic evaluation of stillbirth using formalin-fixed, paraffin-embedded tissue. J Mol Diagn 2013, 15:466-472; http://dx.doi.org/10.1016/j.jmoldx.2013.03.006. The planning committee members and staff have no relevant financial relationships with commercial interests to disclose. The authors have no relevant financial relationship to disclose except for Sarah T. Smith and Joshua D. Schiffman, who received honoraria from Affymetrix for speaking engagements, and Sarah T. Smith, who serves on Affymetrix's Oncology Advisory Board. These relationships were deemed not to be relevant to the educational activity.

Questions #7-10 are based on: Kalman L, Tarleton J, Hitch M, Hegde M, Hjelm N, Berry-Kravis E, Zhou L, Hilbert JE, Luebbe EA, Moxley III RT, Toji L: Development of a genomic DNA reference material panel for myotonic dystrophy type 1 (DM1) genetic testing. J Mol Diagn 2013, 15:518-525; http://dx.doi.org/10.1016/j.jmoldx.2013.03.008. The planning committee members and staff have no relevant financial relationships with commercial interests to disclose. The authors have no relevant financial relationship to disclose except for Madhuri Hegde, who received honoraria as scientific advisor to Genome Quest, RainDance, Tessarar, and Oxford Genetic Technologies. These relationships were deemed not to be relevant to the educational activity.

Questions #11-12 are based on: Dames S, Chou L-S, Xiao Y, Wayman T, Stocks J, Singleton M, Eilbeck K, Mao R: The development of next-generation sequencing assays for the mitochondrial genome and 108 nuclear genes associated with mitochondrial disorders. J Mol Diagn 2013, 15:526-534; http://dx.doi.org/10.1016/j.jmoldx.2013.03.005. The authors of the referenced articles and 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:

- Describe the evaluation of genomic alterations in formalin-fixed, paraffin-embedded (FFPE) stillbirth autopsy tissue.
- Understand molecular inversion probe (MIP) array analysis.
- Describe myotonic dystrophy type 1 (DM1).
- Discuss genetic tests for DM1.
- Describe a new publicly available human cell line-based genomic DNA reference material panel for DM1 genetic testing.
- Understand mitochondrial disorders.
- Describe the mitochondrial and nuclear genes associated with mitochondrial disorders
- Discuss the PCR enrichment methods that are utilized to detect mitochondrial disorders.
1. Stillbirth is defined as the intruterine death of a fetus during the late second or third trimester of pregnancy, but before delivery. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:466-472.]

   a. Stillbirth is defined as the intrauterine death of a fetus with a gestational age >12 weeks.
   b. The number of global stillbirths in 2009 was estimated to be 2.64 million; however, this rate is likely to be underrepresented because of a lack of reporting in developing countries.
   c. In the United States, ~26,000 stillbirths occur each year.
   d. Fetal loss that is unexplained by fetal, placental, maternal, or obstetric influences represents between 25% and 60% of all fetal deaths.

2. Determining the cause of stillbirth is essential for effective patient management, counseling, and determination of recurrence risk. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 466-472.]

   a. Chromosomal analysis using G-banded karyotype has been considered the gold standard for confirming copy number alterations and structural rearrangements in pregnancy loss.
   b. The level of resolution of G-banded karyotyping is 1 to 5 Mb.
   c. Traditional cytogenetic analysis cannot be completed in up to 45% to 60% of stillbirth cases owing to culture failure.
   d. Selective growth of maternal decidua may lead to the cytogenetic diagnosis of a normal female karyotype that is not representative of the true fetal chromosome complement.

3. Postmortem autopsy is a useful means for helping to determine the cause of fetal death. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 466-472.]

   a. The results of fetal autopsy have been reported to change the clinical diagnosis of the cause of fetal death or to yield additional findings in 12% to 26% of cases.
   b. Formalin-fixed, paraffin-embedded (FFPE) tissues processed from fetal autopsy samples offer an alternative to cytogenetic analysis, sidestepping the challenges associated with short-term culture of fetal cells.
   c. An advantage of using FFPE tissues is that analysis can be performed when viable, fresh tissue no longer remains.
   d. FFPE tissues processed from fetal autopsy samples represent an archive of pathologically informative, disease-specific material for genomic profiling.

4. Several obstacles exist when using archival FFPE material for genetic analysis. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 466-472.]

   a. Chemical fixation using formalin causes severe cellular degradation and typically results in reduced nucleic acid quality and quantity.
   b. Chemical fixation using formalin typically results in a higher rate of PCR failure and a lower pass rate on array comparative genomic hybridization (aCGH).
   c. Protein-protein and protein-nucleic acid cross linking that occurs as a result of formaldehyde fixation causes DNA and RNA to be fragmented to an average length of 400 bp.
   d. FFPE tissue age contributes to poor-quality DNA.

5. Molecular inversion probe (MIP) technology is an option for providing high-quality copy number data in FFPE tissue samples. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 466-472.]

   a. The MIP is a nucleic acid probe with an approximately 60-bp footprint.
   b. The 5' and 3' ends of the MIP are complementary to the genomic target of interest.
   c. The MIP's internal region contains a unique barcode tag sequence that identifies the probe, the targeted genomic region, two universal PCR primer sites that are common to all MIPs, and two cleavage sites.
   d. MIPs are designed to have a gap delimited by the hybridized ends of the probes; the gap remains over the target site (the SNP of interest).

6. MIP array analysis of FFPE stillbirth material allows for retrospective evaluation and can be an effective tool for patient management. Based on the referenced Technical Advance article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15: 466-472.]

   a. In the referenced study, one case contained a 15.84 Mb terminal deletion encompassing the ZIC2 gene, which was the second consecutive fetal loss for the mother.
   b. In the referenced study, one case had a 1.8 Mb loss of 17q12 involving the hepatocyte nuclear factor 1B (HNF1B) gene in a fetus noted to have bilateral multicystic dysplastic kidneys.
   c. In the referenced study, four sections (20 μm thick) were taken from archived FFPE tissue blocks.
   d. In the referenced study, microdissection of the FFPE specimens was performed.
7. Myotonic dystrophy type 1 (DM1) is the most common form of adult muscular dystrophy. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:518-525.]
   a. DM1 is a dominantly inherited, multisystem disorder that typically affects skeletal, smooth, and cardiac muscle, the eyes, the brain, and endocrine function.
   b. DM1 is also referred to as Steinert disease.
   c. Penetrance of DM1 is approximately 50% by age 50 years.
   d. There is variable expressivity, and mild cases may be misdiagnosed or undiagnosed.

8. DM1 results from an unstable CTG triplet expansion in the \textit{DMPK} gene. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:518-525.]
   a. \textit{DMPK} encodes a serine-threonine kinase.
   b. The CTG triplet expansion is in the 5' untranslated region of \textit{DMPK}.
   c. \textit{DMPK} is located on chromosome 19q13.3.
   d. Individuals not affected by DM1 have 5 to 34 CTG triplet repeats in leukocyte DNA.

9. DM1 patients with larger CTG repeats tend to have a more severe phenotype. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:518-525.]
   a. The children of patients with \textit{DMPK} alleles in leukocyte DNA with 35 to 49 CTG repeats are asymptomatic.
   b. \textit{DMPK} alleles with CTG repeat expansions >49 lead to a wide spectrum of symptoms that characterize the DM1 phenotype.
   c. \textit{DMPK} alleles >49 CTG repeats are unstable and may expand in length during meiosis, causing offspring to inherit CTG repeats that are longer than those in the parent.
   d. Most DM1 patients display somatic tissue mosaicism in skeletal muscle, heart, and brain, which complicates prediction of the phenotype severity.

10. Clinical laboratories use characterized reference materials for quality assurance purposes. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:518-525.]
    a. The authors developed a genomic DNA reference material panel for DM1 genetic testing using DM1 cell lines.
    b. The three clinical genetics laboratories that participated in the study offer testing for DM1, are located in the United States, and are accredited by the College of American Pathologists.
    c. In addition to using existing DM1 cell lines in the NIGMS repository at the Coriell Institute for Medical Research, cell lines were created by Epstein-Barr virus transformation of B lymphocytes from whole blood samples of consenting patients or their families.
    d. \textit{DMPK} alleles in the samples characterized for the reference panel cover all five DM1 clinical categories.

11. Mitochondrial disorders genetically fall into two classes: mutations in the mitochondrial genome (mtDNA) and genes in the human nuclear genome. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:526-534.]
    a. mtDNA is a maternally inherited, circular, 16,569-bp haploid organelle composed of 37 genes.
    b. It is estimated that up to 400 nuclear genes may be associated with nuclear encoded mitochondrial proteins.
    c. Inheritance of nuclear encoded mitochondrial proteins may be autosomal recessive, dominant, or sex-linked.
    d. Mitochondrial disorders have an overall incidence of 1:5000.

12. Because mitochondrial disorders encompass a wide range of phenotypes and a large number of genes, high-throughput next-generation sequencing (NGS) is an ideal method for variant detection. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2013, 15:526-534.]
    a. NGS allows for a low-cost, comprehensive mitochondrial disorder panel.
    b. NGS offers the ability to sequence at high coverage, allowing for detection of low-level heteroplasmy for mtDNA mutations, which are not easily detected by Sanger sequencing.
    c. Enrichment techniques include long-range PCR (LR-PCR) for the mitochondrial genome and various in-solution and chip-based capture methods for nuclear genes.
    d. The authors effectively used blood samples and other tissue types for the mitochondrial disorder assay.