Articles on next-generation sequencing-assisted DNA-based digital PCR for detection of residual chronic myeloid leukemia, next-generation cystic fibrosis testing of newborns, and ZNF154 DNA hypermethylation analysis as a pan-cancer locus for blood-based diagnostics were selected for the March 2016 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.


Questions #6-8 are based on: Margolin G, Petrykowska HM, Jameel N, Bell DW, Young AC, Elnitski L: Robust detection of DNA hypermethylation of ZNF154 as a pan-cancer locus with in silico modeling for blood-based diagnostic development. J Mol Diagn 2016, 18:283-298; http://dx.doi.org/10.1016/j.jmoldx.2015.11.004

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

- Define the role of BCR-ABL1 in chronic myeloid leukemia (CML).
- Describe the genetics of cystic fibrosis (CF).
- Explain newborn screening (NBS) for CF.
- Explain NBS panels for CF screening and cystic fibrosis transmembrane conductance regulator (CFTR) gene testing.
- Describe the limitations of current next-generation sequencing (NGS) assays.
- Understand the advantages and disadvantages of clinical cancer DNA methylation tests such as methylation arrays and bisulfite amplicon sequencing.

1. The fusion oncogene BCR-ABL1 is the hallmark for chronic myeloid leukemia (CML). Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2016, 18:176-189.]

   a. The BCR-ABL1 tyrosine kinase protein is responsible for the cellular phenotype of CML.
   b. BCR-ABL1 is a rational target for therapy via tyrosine kinase inhibition, a treatment approach that has revolutionized patient outcome.
   c. The measurement of BCR-ABL1 transcripts via quantitative RT-PCR (RT-qPCR) is the most widely used method for monitoring residual disease in patients with CML.
   d. Although tyrosine kinase inhibitors (TKIs) are routinely administered indefinitely, 80% of patients who achieve undetectable BCR-ABL1 by RT-qPCR on imatinib that is sustained for at least two years will remain disease-free after drug discontinuation.
2. Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2016, 18:267-282.]

   a. With an overall incidence of 1 in approximately 1,300 in the United States, CF is among the most common genetic disorders.
   b. A single variant, the deletion of Phe508, accounts for approximately 70% of CF chromosomes worldwide.
   c. In addition to ΔF508, nearly 2,000 other single variants (SNVs), insertions and deletions (indels), and genomic copy number variations (CNVs) have been identified through the Cystic Fibrosis Genetic Analysis Consortium.
   d. CF variants have diverse functional consequences and varying prevalence across ethnicities, emphasizing the need for comprehensive and definitive CFTR analysis in CF molecular testing.

3. Newborn screening for CF enables early detection and management of this common and debilitating genetic disease. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2016, 18:267-282.]

   a. Due to its high prevalence, devastating clinical sequelae, and responsiveness to early intervention, universal newborn screening (NBS) for CF has been implemented across the United States and in many countries worldwide with substantial clinical effect.
   b. Compared to symptomatic presentation, NBS has been shown to accelerate the identification of children at risk of CF by approximately two years.
   c. NBS allows early initiation of nutritional support, respiratory therapy, and prophylaxis against infectious complications.
   d. Early initiation of nutritional support, respiratory therapy, and prophylaxis against infectious complications has long-term benefits, including improved growth, reduced hospitalizations, and extended survival.

4. NBS programs rely on tiered screening strategies. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2016, 18:267-282.]

   a. NBS panels have limited sensitivity, especially in nonwhite ethnic groups.
   b. In California’s diverse population, novel CFTR variations are being found in newborns at rates of 38 per year, in which 30% to 40% appear CF causing.
   c. Asymptomatic infants with one CF-causing mutation and a second mutation of variable clinical consequence who have sweat chloride levels below the diagnostic range for CF in the first months of life are increasingly being reported to have CF as they age.
   d. Until recently, comprehensive CFTR testing was performed exclusively through Sanger sequencing, which is costly, laborious, and time-consuming and, therefore, impractical as a second tier test for most NBS programs.

5. Next-generation sequencing (NGS) technologies have the potential to provide clinical-grade sequence analysis of the entire CFTR gene at less cost than the current screening methods. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2016, 18:267-282.]

   a. Current NGS assays often require relatively large quantities of high-quality genomic DNA derived from fresh peripheral blood samples, which requires an additional appointment and blood draw.
   b. Current screening programs generally collect only DBS from newborns and rely on 3.2 mm punches, which yield low quantities of DNA at variable quality.
   c. To date, only a single NGS CF screening assay has been able to provide accurate results using a single 3.2 mm DBS punch.
   d. Existing NGS CF assays have reported multiplexing capabilities of 5 to 18 specimens per run.

6. Sites that display recurrent, aberrant DNA methylation in cancer represent potential biomarkers for screening and diagnostics. Based on the article, select the ONE statement that is NOT true: [See J Mol Diagn 2016, 18:283-298.]

   a. One in six deaths in the United States is due to cancer, despite an emphasis on prevention, early detection, and treatment that has lowered cancer death rates by 45% in the past two decades.
   b. Further improvements in survival rates are likely to come from improving the limits of detection sensitivity at earlier stages of cancer.
   c. Presently, a diagnosis results from a cadre of screening and diagnostic tools that may include physical examination, radiographic imaging, sputum cytology testing, blood tests, endoscopy, and/or biopsies.
   d. New approaches that rely heavily on genomic information may change future testing strategies.
7. Epigenetic markers are emerging as tools with discriminatory power for disease detection. Based on the article, select the ONE statement that is NOT true: [See J Mol Diagn 2016, 18:283-296.]

a. DNA methylation is a robust epigenetic marker for which a number of commercially available tests have been developed.
b. Newly developed clinical DNA methylation tests detect tissue-specific DNA methylation using clinical specimens and are currently primarily used in breast cancer.
c. One advantage of newly developed clinical DNA methylation tests is marker stability under common storage conditions.
d. Despite DNA methylation's potential as a diagnostic marker, there is a general lack of consensus on this methodology.

8. A pan-cancer hypermethylation signal around a CpG island near human ZNF154 has been detected. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2016, 18:283-296.]

a. In the referenced study, the ZNF154 methylation signal was measured across five tumor types (colon, lung, breast, stomach, and endometrial tumors) using bisulfite amplicon sequencing.
b. An advantage of bisulfite amplicon sequencing is that individual sequence reads are used to quantitate methylation levels of all CpGs within the amplicon while providing quantitative data for each DNA molecule in the pooled sample.
c. A disadvantage of bisulfite amplicon sequencing is that it is difficult to assess quality control.
d. By covering all amplified CpGs, bisulfite amplicon sequencing provides greater resolution of a target region than a methylation array, and reveals patterns of DNA methylation that are useful for distinguishing tumor from normal samples.