ASIP 2019 Journal CME Programs

JMD 2019 CME Program in Molecular Diagnostics

American Society for Investigative Pathology and the Association for Molecular Pathology

The Journal of Molecular Diagnostics, Volume 21, Number 3 (May 2019)

http://www.asip.org/CME/index.cfm

Chhavi Chauhan, PhD, Director of Journal CME Programs

Answers for CME January Questions #1-12

A technical advance on the use of highly multiplexed fluorescence in situ hybridization for comprehensive analysis of copy number changes and two research articles on detecting low-frequency mutations in hematologic malignancies and understanding mitochondrial disease etiology in ethnically diverse population were selected for the May 2019 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:

• Discuss the use of fluorescence in situ hybridization (FISH) in detecting gene copy number changes.
• Discuss the advantages of using FISH over array comparative genomic hybridization (aCGH) and next-generation sequencing (NGS) for diagnosis and management of cancer.
• Discuss the importance of identification of low-frequency genetic variants and their implications in hematological malignancies.
• Discuss the advantages of using molecular barcoded NGS in clinical settings.
• Discuss the uses of NGS in the detection of mitochondrial diseases.

1. Fluorescence in situ hybridization (FISH) is the gold standard technique for the detection of gene copy number changes. Based on the referenced technical advance, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 390–407]
   a. FISH can detect amplifications but not deletions.
   b. FISH can simultaneously evaluate several genes with multiple probes.
   c. FISH is the most widely used technique for detection of copy number changes.
   d. FISH allows absolute copy number quantification at the single-cell level in the context of cancer tissue section architecture.

   a. Besides FISH, array comparative genomic hybridization (aCGH) and next-generation sequencing (NGS) can be used to assess gene copy number changes.
   b. Both aCGH and NGS accurately determine somatic copy number changes in cancer.
   c. The DNA used in both aCGH and NGS is a true representative of tumor cell DNA.
   d. FISH often misses absolute gene spot counts within the tumor cell population.
   a. The assessment of gene copy number is standard of practice regarding the \textit{ERBB2} gene in breast and esophagogastric cancers.
   b. Large panels are now available to simultaneously and accurately assay over 40 well-annotated amplification and deletion events in cancer.
   c. Single-cell sequencing is an alternate and inexpensive approach for assessment of gene copy number changes.
   d. Genomic in situ assays provide high resolution for assessment of gene copy number in cancer.

4. In recent years, NGS technologies have revolutionized the field of clinical genomics. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 471–482]
   a. NGS-based assays are frequently utilized as a guide for management in individual cancer patients.
   b. NGS-based assays have low background error rate.
   c. NGS-based assays allow massively parallel sequencing of hundreds to thousands of genes in a single tube reaction.
   d. NGS-based assays are independent of the evaluation of therapeutic responses or the monitoring of disease progression.

5. The mainstay targeted NGS technologies use either PCR amplification or hybridization capture-based strategies to enrich the target sequences during the preparation of sequencing-ready libraries. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 471–482]
   a. An amplification-based enrichment strategy can be used to sequence hundreds to thousands of genes.
   b. Simple technical validation is sufficient for NGS technologies.
   c. Hybridization capture-based enrichment approaches can be used to sequence tens to hundreds of genes.
   d. Hybridization capture-based approaches are associated with costs that are higher than those for amplification-based NGS assays.

   a. Cancer heterogeneity is independent of the status of low-frequency variants.
   b. Low-frequency variants help study the temporal and spatial clonal evolution of cancer.
   c. Cancer responses to therapy are independent of the status of low-frequency variants.
   d. Disease relapse is independent of the status of low-frequency variants.

7. The emergence of highly sensitive molecular diagnostic approaches, such as droplet digital PCR (ddPCR), has allowed the accurate identification of low-frequency variant alleles in clinical specimens. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 471–482]
   a. The multiplex capabilities of droplet digital PCR for variant detection are flawless.
   b. Sequencing background errors contribute to false-positive variant alleles below a frequency of 2% in conventional NGS experiments.
   c. The lower limit of detection of low-frequency variant by droplet digital–PCR is 0.001%.
   d. ddPCR precludes any requirement of prior knowledge of the variant that is being interrogated.

8. The incorporation of unique molecular barcode sequences during sequencing library preparation has enabled the re-derivation of sequences of original DNA templates. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 471–482]
   a. Molecular barcoded NGS may increase background errors associated with sequencing.
   b. Molecular barcoded NGS may decrease specificity and/or increase limit of detection.
   c. Molecular barcoded NGS may facilitate the identification of de novo variants across multiple regions.
   d. Rational library preparation and sequencing data analytic strategies that integrate molecular barcodes are often applied in the clinical setting.

   a. Mitochondria serve mainly as the energy-producing organelle via oxidative phosphorylation (OXPHOS).
   b. The OXPHOS pathway comprises six protein complexes (Complex I to Complex VI).
   c. The OXPHOS pathway consists of a total of 192 structural subunits.
   d. The proteins in the OXPHOS pathway are encoded solely by the mitochondrial DNA (mtDNA).

10. Mitochondrial disease (MD) is a group of rare inherited disorders with clinically heterogeneous phenotypes. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 503–513]
    a. Over 500 genes, extending beyond the structural OXPHOS genes, have been identified as being involved in MD.
    b. Traditionally, MDs are diagnosed through extensive clinical evaluation, including biochemical analysis, followed by genetic screening for selected mutations.
    c. Recent advances in NGS allow for rapid genetic diagnostics in patients who experience MD, in all patient populations.
    d. It is difficult to detect MD with targeted panel sequencing and unbiased whole-exome sequencing (WES).
11. The genetic diagnosis of MD, and identification of the number of genes involved, has rapidly improved since the first mutations were reported in the late 1980s. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 503–513]
   a. MD criteria scoring are the hallmark of MD diagnostics.
   b. The biochemical evaluation of respiratory chain (RC)/OXPHOS function in tissue is considered obsolete in MD diagnosis.
   c. Of all the NGS-based approaches, exome sequencing is the least helpful in MD diagnosis.
   d. The use of NGS in discriminating primary and secondary MD has been limiting.

12. A retrospective investigation into genetic causes of MD was conducted for 127 patients with clinically suspected and biochemically confirmed RC deficiency in an understudied population (predominantly African). Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 503–513]
   a. The majority of the variants found were classified as pathogenic as they met several mtDNA criteria.
   b. c.1448T>C is a well-known Leber hereditary optic neuropathy–associated mutation, which was found among the observed variants.
   c. The clinical phenotype of the patient with c.1448T>C mutation matched that expected for Leber hereditary optic neuropathy.
   d. Patients with Leber hereditary optic neuropathy exhibit visual failure and optic atrophy.