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CME September Questions # 1-12

Articles on next-generation sequencing for somatic mitochondrial DNA mutations in leukemia, HLA-B*57:01 genotyping real-time PCR, and droplet digital PCR for severe combined immunodeficiency newborn screening were selected for the September 2017 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:

- Describe what leukemic samples are used for the identification of somatic mutations.
- Understand mitochondrial DNA (mtDNA) structure.
- Explain human mtDNA mutations.
- Understand what is necessary to identify mutations in mtDNA.
- Understand that DNA polymorphisms that affect pharmacologic processes.
- Understand the function of and side effects associated with abacavir sulfate (ABC).
- Describe hypersensitivity reactions.
- Understand that HLA-B*57:01 genotyping is clinically beneficial in patients who need antiretroviral therapy.
- Explain HLA-B*57:01 screening technologies.
- Define severe combined immunodeficiency disease (SCID).
- Describe the importance of early detection of SCID in newborns.
- Define droplet digital PCR (ddPCR).

1. Somatic mitochondrial DNA (mtDNA) mutations have been identified in many human cancers, including leukemia. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:711-721.]

   a. Human mtDNA is a 15,669-bp double-stranded, circular DNA molecule.
   b. Human mtDNA encodes 8 polypeptides of the oxidative phosphorylation system, 12 transfer RNAs, and two ribosomal RNAs.
   c. Each cell has several thousand mitochondria, but the exact number varies.
   d. Each mitochondrion contains a variable number of genomes.
2. mtDNA mutations do not follow the pattern of a diploid genome in that a cell may have a single mt genotype (homoplasmy) or multiple mt genotypes (heteroplasmy). Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:711-721.]

a. Sanger sequencing can reliably detect all heteroplasmic variants.

b. mtDNA acquires somatic mutations at a lower rate than nuclear DNA.

c. mtDNA acquires single-nucleotide variants (SNVs) at a lower rate than nuclear DNA.

b. There is no absolutely reliable source of germline mtDNA, especially in older individuals.

3. Blood samples are readily accessible from leukemia patients who achieve morphological remission after treatment. Therefore, a method for the detection of leukemia-associated mtDNA mutations based on comparison with a remission sample may be useful. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:711-721.]

a. The authors studied acute myeloid leukemia (AML) patients.

b. Most patients with AML achieve cytogenetic remission after radiation treatment and will have a suitable remission sample available for study.

b. Most patients with chronic myelogenous leukemia (CML) achieve cytogenetic remission on tyrosine kinase inhibitor treatment and will have a suitable remission sample available for study.

d. The authors developed a next-generation sequencing (NGS) approach to identify leukemia-associated mtDNA mutations using samples from AML patients at diagnosis and in remission after radiation treatment.

4. The authors of the referenced study validated a next-generation sequencing (NGS) approach for the identification of leukemia-associated mtDNA mutations. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:711-721.]

a. Samples were from 26 chronic phase CML patients.

b. The median age of the patients was 42 years and the male/female ratio was 8:18.

c. An empirically determined threshold of 1% was applied to minimize false-positive results.

d. Mutations were called against both non-hematopoietic and remission controls and the overall concordance between the two approaches was 72%.

5. Abacavir sulfate (ABC) is a nucleoside reverse-transcriptase inhibitor used for treating HIV infection. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:742-754.]

a. In approximately 15% to 18% of treated patients, ABC causes hypersensitivity reactions that could be fatal without discontinuation of ABC administration.

b. Since associations between ABC-induced hypersensitivity reactions and the 57:01 allele of human leukocyte antigen, class I, B (HLA-B*57:01) were reported, genetic studies have determined strong and significant links between them.

c. CD3+T cells mediate the hypersensitivity reactions.

d. There are >5,000 HLA-B proteins in humans.

6. Hypersensitivity reactions to ABC seem to be very specific to the HLA-B*57:01 allele. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:742-754.]

a. HLA-B*57:04, HLA-B*57:05, HLA-B*57:11, and HLA-B*58:01 do not induce the hypersensitivity reactions.

b. HLA-B*57:04, HLA-B*57:05, HLA-B*57:11, and HLA-B*58:01 proteins differ in six amino acids only.

c. HLA-B*57:03 and HLA-B*58:01 are different from HLA-B*57:01 in only three amino acid residues among 362 residues of the full-length protein.

d. All of the polymorphic amino acids locate to the antigen-binding cleft of HLA-B*57:01.

7. HLA-B*57:01 genotyping is clinically beneficial in patients who need antiretroviral therapy, by reducing the risk for ABC-driven hypersensitivity reactions. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:742-754.]

a. ABC binds to the cleft of HLA-B*57:03.

b. The complex of ABC and HLA-B*57:03 binds to self-peptides, and presentation of the self-peptides through HLA-B*57:03 induces the hypersensitivity reactions.

c. In terms of cost-effectiveness, genotyping before ABC administration is beneficial.

d. Patients should be screened for HLA-B*57:03 before ABC administration, and ABC is contraindicated in patients who harbor HLA-B*57:03.
8. Several HLA-B*57:01 screening technologies have been developed. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:742-754.]

   a. HLA-B*57:01 screening technologies include flow cytometry, sequence-based typing, sequence-specific oligonucleotide (SSO) probe hybridization-based typing, sequence-specific primer (SSP) PCR, and real-time PCR.
   b. Sequence-specific primer (SSP) PCR allows for the simultaneous detection of several HLA genotypes.
   c. Sequence-specific oligonucleotide (SSO) probe hybridization-based typing followed by gel electrophoresis requires only minimal laboratory equipment.
   d. Real-time PCR—based detection technologies had more sensitivity than previously developed technologies.


   a. SCID is a group of inherited immunodeficiencies characterized by T- and B-cell lymphopenia.
   b. The overall incidence of SCID is reported to be approximately 1:75,000.
   c. Detection of SCID by age 10 years is optimal.
   d. SCID infants treated by hematopoietic stem cell transplantation within the first few months of life have survival rates of approximately 50%.

10. Given the severity of SCID-related symptoms and improved outcomes after an early diagnosis, newborn screening (NBS) fulfills classic Wilson-Jungner screening criteria and was deemed a suitable candidate for NBS by the United States Department of Health and Human Services in May, 2010. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:755-765.]

    a. To date, only one-third of the states have NBS screening programs in place.
    b. NBS for SCID has been evaluated by studies in North America; however, screening programs have not yet been initiated in other continents.
    c. Current SCID screening methods exploit T-cell receptor excision circles (TRECs) that are a DNA by-product of T-cell receptor recombination and reflect T-cell maturity.
    d. TREC levels increase with increasing age.

11. A sensitive method to detect very low concentrations of a DNA target within a single analysis is droplet digital PCR (ddPCR). Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:755-765.]

    a. With ddPCR, a single DNA sample is partitioned into several hundred uniform-size droplets, subsequently amplified by real-time PCR (qPCR), and signal from a fluorescently labeled probe within each droplet is recorded as either positive or negative depending on the presence or absence of a target’s PCR product.
    b. With ddPCR, standard curves of reference standards are essential to determine an absolute concentration determination for a DNA target.
    c. Hundreds of replicate measurements of each sample occur during each ddPCR run.
    d. The application of differentiated labeled fluorescent probes allows for multiplexing PCR targets, such as TRECs and a control, within a single DNA tube and from the same dried blood spot punch.

12. The authors of the referenced article used an automated DNA extraction process. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:755-765.]

    a. DNA was eluted within four hours.
    b. The ddPCR section of the method was completed on the first day.
    c. The ddPCR lower limit of quantitation was 14 TREC copies/μl and the limit of detection was 4 TREC copies/μl.
    d. Testing 29 infants with known lymphocyte profiles resulted in a sensitivity of 98.9% and a specificity of 85% at TRECs <25 copies/μl.