A Perspective on methods-based proficiency testing in molecular genetic pathology and research articles on the molecular
diagnosis of extraskeletal myxoid chondrosarcoma, pathogenicity evaluation of BRCA1 and BRCA2 unclassified variants, and
automated blood group genotyping were selected for the May 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.

Questions #1-3 are based on: Schrijver I, Aziz N, Jennings LJ, Richards CS, Voelkerding KV, Weck KE: Methods-based

M, Gambarotti M: Diagnostic utility of molecular investigation in extraskeletal myxoid chondrosarcoma. J Mol Diagn 2014,

Questions #7-9 are based on: Santos C, Peixoto A, Rocha P, Pinto P, Bizarro S, Pinheiro M, Pinto C, Henrique R, Teixeira
MR: Pathogenicity evaluation of BRCA1 and BRCA2 unclassified variants identified in Portuguese breast/ovarian cancer

Questions #10-12 are based on: Paris S, Rigal D, Barlet V, Verdier M, Coudurier N, Bailly P, Brès JC: Flexible automated
platform for blood group genotyping on DNA microarrays. J Mol Diagn 2014, 16:335-342; http://dx.doi.org/10.1016/j.jmoldx.2014.02.001

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

- Discuss proficiency testing (PT) and its role in quality assurance.
- Understand methods-based proficiency testing (MBPT) as a subset of PT.
- Explain the changes observed in areas of medicine to which molecular diagnostic testing is applied given the
  adoption of sequenced-based clinical testing.
- Describe extraskeletal myxoid chondrosarcoma (EMC).
- Define the classification of EMC.
- Discuss the use of cytogenetics and molecular genetics as ancillary techniques to support an EMC diagnosis.
- Describe the limitations of antibody-based agglutination.
- Understand the advantages of DNA-typing assays for antigen screening.
- Discuss BRCA1 and BRCA2 germline mutations in hereditary breast/ovarian cancer (HBOC) syndrome.
- Understand the importance of BRCA1 and BRCA2 variants of uncertain significance (VUS).

1. Proficiency testing (PT) is intended to be an external measure of clinical laboratory quality. Based on the
   referenced Perspective, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:283-287.]
   
   a. In the United States, PT is a requirement of accreditation by the Centers for Medicare and Medicaid Services.
   b. PT is part of a quality assurance program to verify the efficiency and speed of laboratory testing.
   c. Laboratories in the United States are certified under the Clinical Laboratory Improvement Amendments (CLIA) and
      accredited by professional organizations with deemed status, such as the College of American Pathologists (CAP).
   d. Participation in external quality assessment (EQA) may be through CAP PT programs or through another proficiency
testing provider accepted by CLIA.
2. Methods-based proficiency testing (MBPT) is a subset of overall PT and refers to an EQA approach that is based on method, rather than on each individual analyte tested. Based on the referenced Perspective, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:283-287.]

a. MBPT is already well established for several pathology subspecialty areas.

b. CAP offers a variety of PT products that are methods based, for example, in cytogenetics, flow cytometry, and immunohistochemistry.

c. The concept of MBPT complies with federal laboratory regulations.

d. MBPT in molecular diagnostics currently includes molecular cancer testing, in addition to inherited genetic conditions.

3. With the rapid adoption of sequenced-based clinical testing, the number of disease-causing variants in almost every area of medicine to which molecular diagnostic testing is applied is growing and is projected to expand. Based on the referenced Perspective, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:283-287.]

a. For inherited diseases, our earlier understanding of a single gene mutation for a single disease has evolved with the use of sequenced-based clinical testing.

b. In genome analysis, the number of variants per person is approximately three million.

c. For exomes, the number of variants per person approximates 50,000.

d. With the use of next generation sequencing (NGS), disease-causing mutations will outnumber the handful of genetic mutations that are currently typically tested in clinical laboratories.

4. Extraskeletal myxoid chondrosarcoma (EMC) is a rare mesenchymal tumor. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:314-323.]

a. EMC accounts for <10% of soft tissue sarcomas.

b. EMC rarely occurs in bone.

c. EMC is mainly a tumor of adults, with the median age in the sixth decade.

d. EMC is quite rare in children and adolescents.

5. EMC has been considered a cartilaginous neoplasm because of some morphological findings that suggest chondroid differentiation. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:314-323.]

a. Generally, immunohistochemical studies are not helpful in establishing the diagnosis of EMC but may be useful to exclude other entities.

b. In contrast to other cartilaginous neoplasms, only a few EMCs actually express vimentin.

c. EMC is classified as a tumor of uncertain differentiation in the most recent edition of the World Health Organization Classification of Tumors of Soft Tissue and Bone.

d. On the basis of the results of immunohistochemical and ultrastructural investigations, EMC may have neural or neuroendocrine differentiation.

6. Cytogenetic and molecular genetic studies of EMC have found four pathogenetically relevant chromosome translocations. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:314-323.]

a. The most common translocation t(9;22)(q22;q12), found in approximately 25% of cases, results in a fusion of EWS RNA-binding protein 1 gene (EWSR1) at 22q12 to the nuclear receptor subfamily 4, group A, member 3 (NR4A3) gene at 9q22.

b. Fusion of the EWSR1/NR4A3 chimeric transcripts produces different fusion variants, depending on the breakpoint in the genes.

c. The main variant of EWSR1/NR4A3 chimeric transcripts is the type 1 fusion in which EWSR1 exon 14 is fused to exon 4 of NR4A3.

d. The fusion protein of EWSR1/NR4A3 chimeric transcripts consists of the NH2-terminal transactivation domain of EWSR1 linked to the entire NR4A3 protein.

7. Germline deleterious mutations of BRCA1 and BRCA2 originate the hereditary breast/ovarian cancer (HBOC) syndrome. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:324-334.]

a. Carriers of the BRCA1 and BRCA2 inactivating mutations have an estimated lifetime risk of breast cancer of 65%, and the lifetime risk of ovarian cancer is 33% for BRCA1 carriers and 50% for BRCA2 carriers.

b. Although the magnitude of risk varies with the population and the study design, the finding of a deleterious germline mutation is crucial for the correct clinical management of HBOC families.

c. Finding a germline mutation allows identification of relatives who require increased surveillance and/or prophylactic interventions, as well as those who are not at increased cancer risk.

d. Genetic testing of the index case can identify variants of uncertain significance (VUS), also called unclassified variants, usually missense, silent and intronic variants or in-frame deletions and insertions.

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8. **BRCA1** and **BRCA2** unclassified variants constitute a major problem for genetic counseling and follow-up of families with suspected HBOC syndrome. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:324-334.]

   a. According to software analysis of splicing predictions, four variants that affect highly conserved regions of splicing induce disruption of intron-exon junctions.
   b. **BRCA2** c.8488-1G>A causes the abolishment of the normal splice site and also generates a new splice site with a high score.
   c. **BRCA1** c.4484G>T is a splicing mutation affecting the last nucleotide of **BRCA1** exon 14.
   d. The **BRCA2** c.682-2A>C mutation affects the highly conserved region of the acceptor splice site, induces aberrant splicing with the production of two out-of-frame transcripts and segregates with the disease in the family, and is considered a pathogenic mutation.

9. The intronic or exonic variants that are identified in the exon-intron boundaries of **BRCA1** and **BRCA2** may disrupt the splicing capacity, due to the highly conserved nature of these regions, but the effect is not predictable from genomic sequence alone. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:324-334.]

   a. The **BRCA2** c.2T>G variant causes the disruption of the translation initiation codon.
   b. In the Breast Cancer Information Core (BIC) database, **BRCA2** c.2T>G is described as clinically important, but no additional data is presented.
   c. **BRCA2** c.2T>G abolishes the normal initiation codon of the BRCA2 protein and the closest ATG is in exon 2 leading to an in-frame protein.
   d. The **BRCA2** c.2T>G variant was identified in a family with four affected members; it segregates in three of them and does not segregate in the daughter with breast cancer diagnosed at age 46 years.

10. The standard method of phenotyping for red blood cell (RBC) antigens is antibody-based agglutination. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:335-342.]

    a. Antibody-based agglutination involves long procedure duration.
    b. Antibody-based agglutination involves limited range of antigen testing.
    c. In the French Blood Service, blood donation qualification laboratories test all blood donations for ABO, Rhesus, KEL, MNS3, and MNS4.
    d. Conventional hemagglutination is ill suited to high-throughput blood group phenotyping.

11. Development of DNA-typing assays for antigen screening in blood donation qualification laboratories promises to enable blood banks to provide optimally matched donations. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:335-342.]

    a. Many low-throughput methods allow point-by-point identification of single nucleotide polymorphisms (SNPs).
    b. Low-throughput methods are unsuitable for large-scale genotyping and thus for routine blood donor screening.
    c. Large-scale blood group genotyping is possible using several commercially available assays in various formats.
    d. The currently available commercial assays are fully automated and allow simultaneous detection of a large number of SNPs in a single reaction.

12. High-throughput DNA-typing could have a number of applications in blood banking. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:335-342.]

    a. High-throughput DNA-typing would greatly facilitate support for chronically transfused patients at high risk of alloantibody production, such as patients with sickle cell disease, thalassemia, or autoimmune hemolytic anemia.
    b. In association with conventional serological methods for detection of RBC antigens, routine DNA-based methods blood donor screening would improve transfusion safety by optimizing donor/recipient genocompatibility.
    c. For small batch production using the 96-well format system, the cost of genotyping, including genomic DNA extraction, labor, and equipment, is less than $1.20 per SNP for a multiplex set of 12 SNPs.
    d. A drastic reduction in cost per SNP could be achieved by increasing the number of samples analyzed since the main expenses are microarray fabrication and nucleic acid extraction.