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CME March Questions # 1-12

An article and associated Commentary on targeted next-generation sequencing (NGS) for personalized lymphoma management and articles on molecular diagnostics of human papillomavirus (HPV) PCR tests of head and neck tumor tissues and on RNA detection methods for *Leishmania* were selected for the **March 2018 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 #1-4 are based on: Hung SS, Meissner B, Chavez EA, Ben-Neriah S, Ennishi D, Jones MR, Shulha HP, Chan FC, Boyle M, Kridel R, Gascoyne RD, Mungall AJ, Marra MA, Scott DW, Connors JM, Steidl C: Assessment of capture and amplicon-based approaches for the development of a targeted next-generation sequencing pipeline to personalize lymphoma management. J Mol Diagn 2018, 20:203-214; https://dx.doi.org/10.1016/j.jmoldx.2017.11.010 and associated Commentary by Ohgami RS, Rosenwald A, Bagg A: Next-generation sequencing for lymphomas: perfecting a pipeline for personalized pathobiologic and prognostic predictions. J Mol Diagn 2018, 20:163-165; https://dx.doi.org/10.1016/j.jmoldx.2018.01.002

Questions #5-8 are based on: Huho AN, Yadak N, Bocklage TJ, Yang S: Evaluation of diagnostic utility of a high-risk human papillomavirus PCR test on formalin-fixed, paraffin-embedded head and neck tumor tissues. J Mol Diagn 2018, 20:232-239; https://dx.doi.org/10.1016/j.jmoldx.2017.11.008

Questions #9-12 are based on: Eberhardt E, Van den Kerkhof M, Bulté D, Mabille D, Van Bockstal L, Monnerat S, Alves F, Mbui J, Delputte P, Cos P, Hendrickx S, Maes L, Caljon G: Evaluation of a pan-*Leishmania* spliced-leader RNA detection method in human blood and experimentally infected Syrian golden hamsters. J Mol Diagn 2018, 20:253-263; https://dx.doi.org/10.1016/j.jmoldx.2017.12.003

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

- Describe the demographics of lymphomas.
- Understand the basis of hybrid-capture and amplicon-based approaches for targeted next-generation sequencing (NGS).
- Describe clinically applicable sequencing-based assays in lymphoma cases.
- Describe the genetic profile of mutations in diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL).
- Discuss the role of high-risk human papillomaviruses (HR-HPV) in head and neck squamous cell carcinomas (HNSCC).
- Describe immunohistochemical and hybridization methods for detection of HPV.
- Discuss the advantages of HPV detection methods in formalin-fixed, paraffin-embedded (FFPE) tissues.
- Describe leishmaniasis and the parasites that cause it.
- Define spliced-leader RNAs.
- Understand the performance of a new real-time quantitative PCR (qPCR) assay for leishmaniasis.

1. Lymphoid cancers can be regularly cured in a subset of patients, indicating the potential to improve outcomes if currently available treatments are optimized and strategically applied. Based on the referenced article and associated Commentary, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:203-214 and J Mol Diagn 2018, 20:163-165.]

a. Lymphoid cancers disproportionally affect older patients.
b. In Canada, lymphoid cancers are the fourth most common cancer.

c. Fortunately, the last five decades have witnessed a decrease in incidence of lymphoid cancers.
d. The three most common lymphomas are diffuse large B-cell lymphoma (DLBCL), Hodgkin lymphoma, and follicular lymphoma (FL).

2. Targeted next-generation sequencing (NGS) has become a more frequent option in routine clinical practice. Based on the referenced article and associated Commentary, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:203-214 and J Mol Diagn 2018, 20:163-165.]

a. The first genetic abnormalities of B-cell lymphomas were revealed more than three decades ago largely based on karyotypic analyses such as *MYC* translocation in Burkitt lymphoma.
b. Advantages of hybrid-based capture approaches are a simplified workflow and requirement for smaller amounts of DNA.
c. Amplicon-based approaches are less likely to miss mutations and perform better with respect to sequencing complexity and uniformity of coverage.
d. Recent examples of clinically applicable sequencing-based assays include the development of the m7-FLIPI in DLBCL.

3. The authors of the referenced article developed a 32-gene NGS panel for personalized lymphoma management. Based on the referenced article and associated Commentary, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:203-214 and J Mol Diagn 2018, 20:163-165.]

a. A cohort of 260 of patients was studied.
b. The superiority of amplicon sequencing over hybrid-capture sequencing was demonstrated in formalin-fixed, paraffin-embedded (FFPE) tissues and blood samples.
c. The 32-gene panel includes exons spanning 263 kb of genomic sequence.
d. Sanger sequencing validated 100% of somatic mutations called by capture-sequenced lymphoma cases having an allele frequency of >5% and with variant read support of at least 20 reads.

4. Subtype-specific mutational profiles derived from the 32-gene panel were highly representative of published profiles in FL, DLBCL, and chronic lymphocytic leukemia (CLL). Based on the referenced article and associated Commentary, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:203-214 and J Mol Diagn 2018, 20:163-165.]

a. The most commonly detected mutation in both FL and DLBCL was *EZH2*.
b. Missense mutations were predominantly found in known tumor-suppressor genes.
c. Truncating mutations were predominantly found in driver genes such as *MYD88*.
d. The referenced study was designed to address point mutations and insertion/deletions.

5. High-risk human papillomavirus (HR-HPV) is an etiologic agent in a subset of head and neck squamous cell carcinomas (HNSCC). Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:232-239.]

a. In oropharyngeal squamous cell carcinomas (OPSCC), transcriptionally active HR-HPV was found in nearly half of the cases.
b. HPV-related HNSCCs are molecularly and morphologically similar to smoking- and alcohol-related HNSCCs.
c. Prognostically, HPV-related HNSCCs have a greater likelihood for local recurrence.
d. Numerous clinical trials have shown the benefit for de-escalation therapy to decrease treatment-related morbidity in HPV-positive OPSCCs.

6. Many different methods are available to test for HPV in OPSCC. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:232-239.]

a. The most widely used methods for HR-HPV detection are immunohistochemistry (IHC) for p16 and DNA *in situ* hybridization (DNA-ISH).
b. IHC for p16 has a high sensitivity but a low specificity in the oropharynx.
c. IHC for p16 has a high specificity outside the oropharynx.
d. HPV DNA-ISH is less costly than IHC for p16.

7. The authors of the referenced article developed and validated a modified PCR method to detect HR-HPV in FFPE HNSCC tissues. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:232-239.]

a. A total of 175 HNSCC FFPE blocks were collected randomly from the pathology archives of the University of New Mexico Hospital.
b. To determine the analytical sensitivity of the test, a serial dilution of lysate from a highly positive sample (>95% p16-positive tumor and HPV-16 PCR positive) was tested.
c. The last dilution of the highly positive sample described in (b) in which HPV-16 was detectable by PCR corresponded to approximately 16 cells/mL.
d. All FFPE samples collected within 10 years were tested successfully.

8. The validated PCR method to detect HR-HPV described by the authors proves the feasibility of performing HR-HPV PCR on paraffin-embedded fine needle aspirate materials. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:232-239.]

a. A drawback of the modified HR-HPV PCR test on FFPE samples is that it must be run independently of regular cervical swab samples, thus interfering with the routine workflow of cervical HR-PV screening.
b. Processed tissue lysates were stable for up to 45 days at room temperature.
c. High viral quantities within neoplastic cells may lead to cross-contamination of samples between microtome sectioning steps or during the lysate preparation process if care is not taken.
d. The turnaround time of 12 hours was equivalent to other confirmatory tests.

9. Leishmaniasis is a family of related diseases caused by various species of protozoan *Leishmania* parasites. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:253-263.]

a. Phylogenetic analysis identified three different subgenera.
b. *Leishmania* subgenera predominantly occur in the New World.
c. *Viannia* subgenera predominantly occur in the Old World.
d. Manifestations of leishmaniasis range from cutaneous to disseminated cutaneous, mucocutaneous, and visceral clinical signs.

10. Visceral leishmaniasis (VL) is a neglected tropical disease. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:253-263.]

a. According to the World health Organization (WHO), there are nearly 15,000 deaths annually.
b. All VL patients exhibit fever, weight loss, anemia, and hepatosplenomegaly, regardless of parasite burden.
c. VL patients with low parasite burdens may remain asymptomatic.
d. Fortunately, treatment of VL patients with amphotericin B is universally successful.

11. Most PCR methods for the detection of *Leishmania* target minicircle kinetoplast DNA (kDNA). Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:253-263.]

a. kDNA is present in copy numbers up to 10^2 parasites/mL.
b. Heterogeneity of kDNA minicircles has precluded the development of a pan-*Leishmania* real-time quantitative PCR (qPCR) assay.
c. The kDNA PCR assays perform well on *L donovani* in dogs.
d. The kDNA PCR assays can detect 10^{-4} *L. infantum*-parasites in mice.

12. The authors of the referenced article developed a new qPCR method for viable *Leishmania* quantification. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:253-263.]

a. The highly expressed spliced-leader (SL) mini-exon RNA sequence is 39-bp long.
b. SLs are non-coding sequences that are attached during *cis*-splicing to the 3' end of mRNAs.
c. The SL sequence was conserved in all species of the *Leishmania* subgenus but not the *Viannia* subgenus.
d. The SL RNA detection method performed better in cutaneous *Leishmania* species than in visceral species.