

ASIP 2017 Journal CME Programs

JMD 2017 CME Program in Molecular Diagnostics

American Society for Investigative Pathology *and the*
Association for Molecular Pathology

The Journal of Molecular Diagnostics, Volume 19, Number 1 (January 2017)

www.asip.org/CME/journalCME.htm

Mark E. Sobel, MD, PhD, Director of Journal CME Programs

CME November Questions # 1-12

A Review article on applications of DNA-based liquid biopsy for central nervous system neoplasms, an article on microsatellite instability in endometrioid and colorectal cancers, and an article on deamination effects in next-generation sequencing were selected for the **January 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.

Questions #1-5 are based on: Wang J, Bettgowda C: Applications of DNA-based liquid biopsy for central nervous system neoplasms. *J Mol Diagn* 2017, 19:24-34; <http://dx.doi.org/10.1016/j.jmoldx.2016.08.007>.

Questions #6-10 are based on: Wang Y, Shi C, Eisenberg R, Vnencak-Jones CL: Differences in microsatellite instability profiles between endometrioid and colorectal cancers: A potential cause for false-negative results. *J Mol Diagn* 2017, 19:57-64; <http://dx.doi.org/10.1016/j.jmoldx.2016.07.008>.

Questions #11-12 are based on: Kim S, Park C, Ji Y, Kim DG, Bae H, van Vrancken M, Kim DH, Kim KM: Deamination effects in formalin-fixed, paraffin-embedded tissue samples in the era of precision medicine. *J Mol Diagn* 2017, 19:137-146; <http://dx.doi.org/10.1016/j.jmoldx.2016.09.006>.

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

- Explain the complex nature of gliomas.
- Describe biomarker detection and use in oncology.
- Define circulating tumor DNA (ctDNA).
- Describe circulating tumor cells (CTCs).
- Describe extracellular vesicles (EVs) and understand the classification of EVs.
- Define Lynch syndrome (LS).
- Describe mismatch repair (MMR) genes and their role in microsatellite instability (MSI).
- Understand LS screening methods.
- Understand endometrioid cancer (EMC) screening using the MSI PCR assay.
- Describe cytosine deamination.
- Understand deamination-induced nucleotide changes.

1. As more is understood about the genetic basis of gliomas, it has become increasingly clear that they are a dynamic and complex tumor, difficult to neatly categorize. Based on the referenced Review, select the ONE best TRUE statement: [See *J Mol Diagn* 2017, 19:24-34.]

- a. A study using single-cell RNA sequencing found that subpopulations of cells with each of the six gene-expression signatures detailed by The Cancer Genome Atlas (TCGA) can exist within a single glioblastoma (GBM) lesion.
- b. A report found that in more than 60% of low-grade glioma cases, the majority of mutations present at the time of diagnosis were not found at the time of tumor recurrence.
- c. Pseudoresponse, which can be seen in up to 30% of cases after chemotherapy and radiation therapy, refers to the false appearance of tumor growth.
- d. Selective pressure applied by chemotherapeutic agents, most notably temozolomide, results in a hypermutated genotype at recurrence.

2. A promising biomarker associated with an improved clinical outcome must provide clinically actionable information that can help guide management and ultimately change outcomes before it can enter clinical practice. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:24-34.]

- a. In 2015, the National Comprehensive Cancer Network (NCCN) evaluated the current state of biomarkers in oncology, finding few biomarkers with adequate evidence to incorporate into the standard of care across eight cancer types.
- b. Only four biomarkers met criteria for clinical utility for glioma in a 2011 NCCN report.
- c. Circulating biomarkers, including circulating tumor nucleic acids (ctNAs), circulating tumor cells (CTCs), and extracellular vesicles (EVs), have shown tremendous promise as a type of “liquid biopsy” in oncology.
- d. Although the technical aspects of biomarker detection require further optimization, these tools have already demonstrated their diagnostic, prognostic, and predictive value in neuroblastoma and pancreatic cancer.

3. DNA fragments circulating freely in blood, or cell-free DNA, have been described in the literature for nearly 70 years. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:24-34.]

- a. It has become clear that all cells shed fragments of nucleic acid, typically 50 to 125 base pairs in length.
- b. With disease states, such as cancer, the production of DNA fragments outpaces clearance mechanisms and results in the accumulation of circulating tumor DNA (ctDNA) in the circulation.
- c. As a biomarker, ctDNA displays several favorable features; its half-life of more than 3 hours makes it ideal for studying dynamic changes in tumor homeostasis.
- d. ctDNA carrying tumor-specific mutations can represent as little as 1% and as much as 70% of the total cell-free DNA in the circulation..

4. Tumor cells can circulate in the bloodstream either alone or in clusters, and are thought to be shed from the primary tumor or from metastatic sites into the circulation. Based on the referenced Review, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:24-34.]

- a. Few studies have examined the half-life of CTCs in the circulation, but in one study of prostate cancer and another of breast cancer, it was estimated to be approximately 0.5 hours.
- b. The frequency of CTCs in blood is estimated to be one per 10^7 to 10^{11} white blood cells per mL of blood.
- c. The ideal method of CTC isolation would involve using a marker expressed consistently and exclusively on CTCs and not on hematologic cells.
- d. CTCs can be positively enriched for expressing epithelial cell-surface markers, most commonly by immunomagnetic capture using beads coated in cytokeratin.

5. EVs, which contain DNA as well as other macromolecules, can be categorized into exosomes, microvesicles, and apoptotic bodies on the basis of size, morphology, and mechanism of generation. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:57-64.]

- a. EVs have a long half-life and are released at the same rate by normal cells under physiological conditions and by tumor cells.
- b. Exosomes are typically 20 to 50 nm in diameter.
- c. Microvesicles are 50 to 1000 nm in diameter.
- d. Apoptotic bodies are the largest subcategory of EVs and range from 1000 to 3000 nm.

6. Colorectal (CRCs) and endometrioid (EMCs) cancers in patients with Lynch syndrome (LS) exhibit microsatellite instability (MSI). Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:57-64.]

- a. LS is a heterogeneous disorder exhibiting reduced penetrance, differences in age of onset, and variability in expression.
- b. LS is an autosomal dominant disorder with a disease prevalence of 1 in 220.
- c. About 1% to 2% of CRCs and 2% to 3% of EMCs are due to LS.
- d. In LS patients, the lifetime risks are 30% to 50% for CRC and 20% to 40% for EMC in women.

7. LS is caused by germline mutations. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:57-64.]

- a. LS is caused by one of six mismatch repair (MMR) genes.
- b. Germline mutations in MMR result in defective machinery that leads to microsatellite instability (MSI) throughout the genome and gives rise to tumors.
- c. Only LS-related cancers can manifest MSI.
- d. MSI tumors are associated with a poor prognosis.

8. Many institutions have adopted an algorithm for universal screening of MSI in all newly diagnosed CRCs and EMCs to identify patients with potential LS. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:57-64.]

- a. A method of clinical screening for LS is MSI detection by PCR with template DNA extracted from blood.
- b. A method of clinical screening for LS **immunohistochemical** (IHC) staining uses antibodies directed against EPCAM proteins in tumor tissue sections.
- c. MSI is characterized by the expansion or contraction of DNA sequences through the insertion or deletion of repeated DNA sequences.
- d. If MSI is detected at $\geq 50\%$ of the loci analyzed, the tumor is considered to have a high frequency of MSI (MSI-H).

9. Low frequency of MSI (MSI-L) or microsatellite stable (MSS) status greatly reduces the likelihood of LS in a patient. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:57-64.]

- a. The MSI PCR assay measures the function of the MMR system and may identify MSI-H cases caused by missense mutations of MMR genes that may not result in the loss of immunoreactivity and thus could be missed by IHC.
- b. MSI PCR assay and IHC have a reported sensitivity of 95% to 97%.
- c. Initially, MSI testing was performed using three mono- and four-dinucleotide polymorphic DNA markers, as presented at a National Cancer Institute 1998 workshop.
- d. Currently, the majority of diagnostic laboratories use a commercial kit containing six mononucleotide markers.

10. Although the algorithm for CRC screening may include IHC or MSI or both, it is largely an institutional decision based on cost, expertise, and resources. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19: 19:57-64.]

- a. A study reported that 75% of mutS homolog 6 (MSH)-6 mutant EMCs do not show MSI-H by MSI PCR.
- b. Another study documented that 41% of IHC-deficient EMC tumors were MSS by the MSI PCR assay.
- c. A recent study showed 88% concordance between IHC and the MSI-PCR assay; 10% MSI-H EMCs were missed by IHC, yet only 5% of IHC-deficient EMCs were reported missed by the MSI assay.
- d. The correlation of MSI status with pathologic features of EMCs is controversial.

11. Although formalin-fixed, paraffin-embedded (FFPE) tissue samples are easily accessible, the poor quality of FFPE samples is known to interfere with the output of next-generation sequencing (NGS) studies. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:137-146.]

- a. Deamination of nucleotides causes C:G>T:A changes in FFPE tissue samples.
- b. C:G>T:A is caused by a chemical reaction called guanine deamination.
- c. Deamination-induced nucleotide changes occur with high frequency in both tumor-free and tumor tissues.
- d. The only known cause of deamination is formalin fixation.

12. Attempts have been made to reduce deamination-induced errors in FFPE tissue blocks. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2017, 19:137-146.]

- a. The intracellular enzyme uracil DNA glycosylase (UDG) specifically repairs deamination-induced changes at CpG dinucleotide sequences.
- b. The authors of the referenced article concluded that prolonged fixation for >3 days significantly reduced the NGS quality parameters in all of the samples tested.
- c. Samples fixed in acidic pH of 5 had the same high quality NGS quality parameters as samples fixed at neutral pH.
- d. UDG treatment reduced deamination-induced nucleotide changes.