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CME September Questions # 1-12

A Technical Advance describing miRNA recovery from urine and research articles on mutation analysis of pseudomyxoma peritonei and measurement of mitochondrial DNA content were selected for the **September 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 #1-3 are based on: Armstrong DA, Dessaint JA, Ringleberg CS, Hazlett HF, Howard L, Abdulla MAK, Barnaby RL, Stanton BA, Cervinski MA, Ashare A: Pre-analytical handling conditions and small RNA recovery from urine for miRNA profiling. J Mol Diagn 2018, 20:565-571; http://dx.doi.org/10.1016/j.jmoldx.2018.04.003.

Questions #4-7 are based on: Pengelly RJ, Rowaiye B, Pickard K, Moran B, Dayai S, Wapper W, Mirnezami A, Cecil T, Mohamed F, Carr N, Ennis S: Analysis of mutation and loss of heterozygosity by whole-exome sequencing yields insights into pseudomyxoma peritonei. J Mol Diagn 2018, 20:612-620; http://dx.doi.org/10.1016/j.jmoldx.2018.05.002.

Questions #8-12 are based on: Hsieh AYY, Budd M, Deng D, Gadawska I, Côté HCF: A monochrome multiplex real-time quantitative PCR assay for the measurement of mitochondrial DNA content. J Mol Diagn 2018, 20:635-642; http://dx.doi.org/10.1016/j.jmoldx.2018.05.001

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

- Understand the potential of extracellular vesicles (EVs) as sources of biomarkers.
- Define miRNAs.
- Describe the benefits of using urine as a biospecimen for research and diagnostics.
- Describe pseudomyxoma peritonei (PMP).
- Define the genetic abnormalities in appendiceal and colorectal neoplasia.
- Discuss the role of Wnt signaling in colorectal cancer.
- Describe somatic variants in PMP.
- Describe mitochondrial DNA (mtDNA).
- Discuss real-time quantitative PCR (qPCR) for mtDNA measurement.
- Describe the monochrome multiplex qPCR (MMqPCR) assay for mtDNA content and understand its limits.

1. Extracellular vesicles (EVs) are potential sources of biomarkers for multiple diseases. Based on the referenced Technical Advance, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:565-571.]

a. EVs are lipid bilayer vesicles secreted by most cell types.
b. EVs contain molecular constituents of their cell of origin, including miRNAs, which are a class of noncoding RNAs of 15 to 22 nucleotides in length.
c. miRNAs are unstable in EVs within most body fluids.
d. Long noncoding RNAs and mRNAs in EVs have been demonstrated to be more stable than miRNAs.

2. Urine is an optimal biofluid source for clinical analysis and biomarker studies because of its completely noninvasive sampling, high-volume collection, and ease of repeat measurements. Based on the referenced Technical Advance, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:565-571.]

a. Urine samples in the referenced study were collected between the hours of 9 and 11 AM.
b. Donors for the referenced study included healthy volunteers, patients with renal transplant or chronic kidney disease, and individuals heterozygous for *F508del*.
c. Whole urine in this study was defined as spot urine with no centrifugation or filtration steps to eliminate cells and debris.
d. In the referenced study, cell-free urine samples were generated from whole urine by a two-step centrifugation process at 300 x *g* for 15 minutes followed by 1000 x *g* for 5 minutes to eliminate cells and debris.

3. Some pre-analytical handling conditions are better for small RNA recovery and miRNA profiling than others. Based on the referenced Technical Advance, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:565-571.]

a. Urine can be held at or near body temperature for up to 24 hours before storage for optimal miRNA profiling.
b. It is not necessary to spin out cells and debris from whole urine immediately after collection for optimal miRNA profiling.
c. The optimal handling condition for urine is sample storage at a room temperature of 20^{o}C and processing as soon as possible after collection.
d. For optimal miRNA recovery, a 10-minute 37^{o}C sample equilibration is recommended for freeze-thaw whole urine samples.

4. Recent advances in the treatment of pseudomyxoma peritonei (PMP) have increased the need for clinically useful biomarkers to aid management decisions and as prognostic outcome indicators. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:612-620.]

a. The estimated incidence of PMP is about four million per year.
b. If untreated, PMP progresses within the abdomen, ultimately leading to death.
c. The cause of PMP is predominantly from a ruptured mucinous tumor of the ovary.
d. Among mucins that are overproduced in PMP, MUC4 is the most common.

5. Important differences exist between the genetic abnormalities observed in appendiceal and colorectal neoplasia. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:612-620.]

a. Mucinous tumors of the appendix and PMP of appendiceal origin commonly contain mutations of *KRAS* and *BRAF*.
b. Mutations of *TP53* are associated with low-grade disease.
c. Immunohistochemical overexpression of p53 is associated with a good prognosis.
d. *GNAS* mutations are common in mucinous tumors of the appendix and PMP of appendiceal origin but are rare in colorectal neoplasia.

6. Wnt signaling cascades are important in colorectal cancer. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:612-620.]

a. In the colorectum, the commonest oncogenic pathway is *APC* mutation, leading to abnormalities in the Wnt signaling pathway.
b. Mutant *APC* is commonly found in appendiceal mucinous tumors.
c. Nuclear β-catenin expression is seen in most appendiceal adencarcinomas.
d. Mucinous appendiceal tumors commonly show the microsatellite instability loss of expression of DNA mismatch repair proteins and *BRAF* mutations typical of the serrated pathway in the colorectum.

7. The authors of the referenced article applied whole-exome sequencing to tumor-normal paired formalin-fixed, paraffin-embedded PMP samples and obtained data adequate for the detection of somatic variants. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:612-620.]

a. *GNAS* pathogenic mutations were identified in four of the five samples studied.
b. *KRAS* pathogenic mutations were identified in four of the five samples studied.
c. Across the five samples studied, 20% of the identified coding mutations were frameshifts and 42% were possible effectors of splicing.
d. There was a substantial excess of C>T transitions in the context of N[C>T]G, consistent with deamination of 5-methylcytosine as the molecular mutation mechanism.

8. Mitochondrial DNA (mtDNA) content is a prevalent biomarker across both clinical and basic research. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:634-642.]

a. mtDNA encodes 19 tRNAs, one rRNA, and several proteins.
b. The copy number of mtDNA per cell is modulated on the basis of the cell's energetic needs as well as the presence of the mtDNA-encoded mitochondrial transcription factor A.
c. Mitochondrial transcription factor A acts on the promoter regions of mtDNA to regulate replication and transcription.
d. Reduced mtDNA content is a hallmark of mitochondrial DNA depletion syndrome, which clinically presents as renal failure.

9. Real-time quantitative PCR (qPCR) is the preferred method of measuring mtDNA content. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:634-642.]

a. The authors of the referenced article implemented a monochrome multiplex qPCR (MMqPCR) technique for the measurement of mtDNA content using SYBR Gold intercalating fluorescent dye.
b. In the referenced study, mtDNA content was defined as the ratio of the copy number of mitochondrial genomes normalized to the copy number of a single-copy nuclear gene.
c. Fragments of the mitochondrial gene for $tRNA^{Phe}$ were used as mtDNA sequences in the MMqPCR assay.
d. Fragments of the gene for β-actin (*ACTB*) were used as nuclear DNA sequences in the MMqPCR assay.

10. The MMqPCR technique relies on the addition of extra nucleotides to the PCR primers. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:634-642.]

a. The addition of extra nucleotides to the PCR primers alters the dissociation temperatures of the two amplicons.
b. Longer GC-rich tags were added to the D-loop MMqPCR primers and shorter tags were added to the *ALB* MMqPCR primers.
c. The combined D-loop/*ALB* signal was acquired at 72^{o}C.
d. Only the D-loop signal was acquired at 88^{o}C.

11. The authors of the referenced study compared the MMqPCR technique with gold-standard monoplex qPCR. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20:634-642.]

a. mtDNA content measurements of human tissues extended linearly from 409 to 7524 copies of D-loop per copy of *ALB*, across a nearly 20-fold range.
b. Lin's concordance correlation coefficient was greater than 0.95.
c. Pearson's correlation coefficient was robust at $r > 0.88$.
d. In subanalyses using whole blood, which is at the low end of the biological range of mtDNA content, there was a narrow fivefold range of linear mtDNA content measurement and a correlation of $r < 0.70$.

12. In qPCR assays, the amount of template DNA must exceed the limit of quantification to attain an accurate measurement. Based on the referenced article, select the ONE best TRUE statement: [See J Mol Diagn 2018, 20: 634-642.]

a. The limit of quantification in the MMqPCR assay was determined to be on the order of 5 nuclear genomes.
b. The mtDNA/nuclear DNA ratio in the DNA assayed was demonstrated to exceed 35 for accurate quantification.
c. Typically, the mtDNA content of a physiologically relevant DNA sample will be substantially >30.
d. Whole blood mtDNA content ranged from 39 to 196, with a median value of 99, similar to reports in the literature of ~100 copies of mtDNA/nuclear DNA.