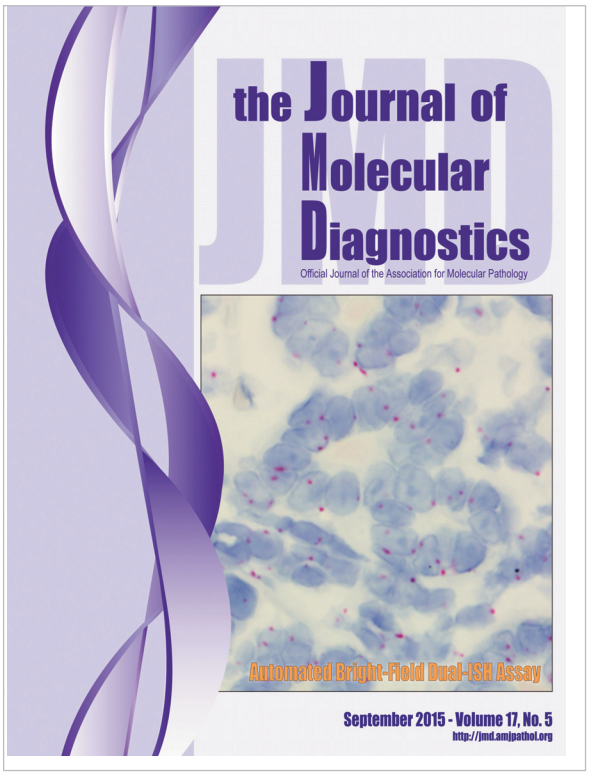
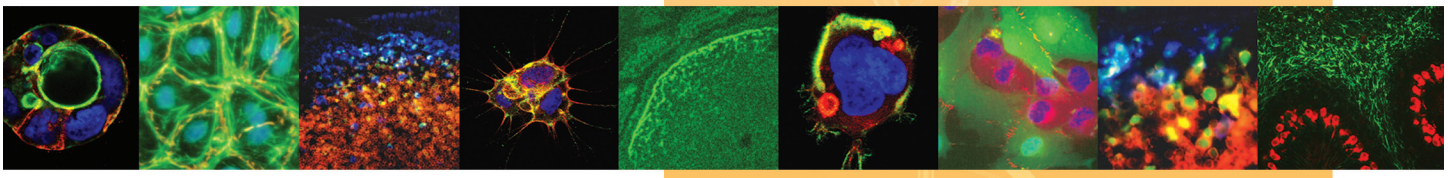


ASIP Journal CME Program

Answer Booklet for the *JMD* 2015 CME Program in Molecular Diagnostics

2015 CME Exam Questions & Answers



For more information on how to participate in future CME programs, visit:

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Program Director: Mark E. Sobel, MD, PhD

the Journal of Molecular Diagnostics

JMD 2016 Journal CME Program
JMD Volume 18, Issues 1-6
Director of Journal CME Programs: Mark E. Sobel, MD, PhD
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2016 Registration Form – *JMD* CME Program			
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SPECIAL INSTRUCTIONS:

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If you are an ASIP member or a non-member of ASIP or AMP, please mail or fax your Registration Form to the ASIP Education Office (301) 634-7990. ASIP Office Address: 9650 Rockville Pike, Suite E-133, Bethesda, MD 20814-3993 (USA)

American Society for Investigative Pathology
Investigating the Pathogenesis of Disease

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JMD CME PROGRAM IN MOLECULAR DIAGNOSTICS

Dear Colleague,

The JMD 2015 CME Program in Molecular Diagnostics was organized as an annual program in which participants were awarded CME credit by successfully answering questions on selected articles in each bimonthly issue of the Journal. The JMD 2015 CME Program in Molecular Diagnostics offered 6 *AMA PRA Category 1 Credit(s)*™ for the successful completion of each bimonthly exam. The American Board of Pathology approved this program for SAM credits (maximum of 36 credits for the year).

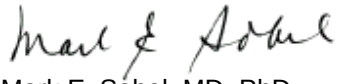
We are pleased to provide the Answer Booklet for the six 2015 CME exams on the following pages.

The JMD 2016 CME Program in Molecular Diagnostics is organized in the same way. Please visit the Journal CME website http://www.asip.org/CME/JMD_2016.cfm for more information. **Members of the Association for Molecular Pathology** (**AMP) and the American Society for Investigative Pathology receive a 40% discount on the subscription fee for the annual program and may receive a maximum of 36 *AMA PRA Category 1 Credit(s)*™ if all 6 bimonthly exams are successfully completed.** You can achieve credit for each exam successfully completed; it is not necessary to complete all 6 exams.

The Journal of Molecular Diagnostics is jointly owned by ASIP and the Association for Molecular Pathology.

I gratefully acknowledge the American Society for Clinical Pathology for joint providership of the ACCME-accredited JMD CME Program in Molecular Diagnostics since July 2012.

Sincerely yours,



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

CONTINUING MEDICAL EDUCATION (CME) INFORMATION

CME Accreditation Statement: This activity ("2015 *JMD* CME Program in Molecular Diagnostics") has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint providership of the American Society of Clinical Pathology (ASCP) and the American Society for Investigative Pathology (ASIP). ASCP is accredited by the ACCME to provide continuing medical education for physicians.

The ASCP designates this journal-based CME activity ("2015 *JMD* CME Program in Molecular Diagnostics") for a maximum of 36 *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Description
The 2015 *JMD* CME Program in Molecular Diagnostics is an annual program consisting of a series of at least 36 questions based on selected articles in the 2015 issues (Volume 17) of *The Journal of Molecular Diagnostics* (*JMD*). Bimonthly exams, consisting of at least 6 questions that are based on selected articles appearing in each issue of the Journal, will be available online on the Journal website for registered participants.

To receive CME credit for this journal-based CME activity, participants must achieve a score of at least 75% on each bimonthly exam and complete a Post-Test Evaluation. All exams must be completed by December 31, 20154 to receive CME credit. Participants will earn 6 *AMA PRA Category 1 Credit(s)*™ for the successful completion of each bimonthly exam (a score of at least 75% of the questions answered correctly for each bimonthly exam).

For more information please contact the ASIP Education Office by phone at (301) 634-7440; email (journalcme@asip.org), or mail your inquiry to 9650 Rockville Pike, Suite E-133, Bethesda, MD 20814.

SAM Credit
The 2015 *JMD* CME Program in Molecular Diagnostics is approved by the American Board of Pathology for up to 36 SAM credits. Physicians should only claim credit commensurate with the extent of their participation in the activity. After successfully completing the bimonthly CME exams as described above, participants may separately apply for SAM credit by completing the SAM application found on the ASIP website (http://www.asip.org/CME/documents/ASIP2015JMDSAMApplication.pdf). All SAM applications must be received in our office by December 31, 2015 for participants to receive SAM credit.

For more information regarding SAM credits, please contact the ASIP Education Office by phone at (301) 634-7440; email (journalcme@asip.org), or mail your inquiry to 9650 Rockville Pike, Suite E-133, Bethesda, MD 20814.

Objective/Target Audience
The objective of the 2015 *JMD* CME Program in Molecular Diagnostics is to increase basic and applied pathology knowledge, focusing on the molecular pathogenesis, diagnosis, prognosis, and the treatment of disease. The 2015 *JMD* CME Program in Molecular Diagnostics is designed to meet the participants' education needs in the physician competency area of Medical Knowledge, as

defined by the Accreditation Council for Graduate Medical Education (ACGME) and the American Board of Medical Specialties (ABMS), and to support participants' lifelong learning towards a goal of promoting patient safety and improving patient care. The program is specifically targeted to pathologists and laboratory professionals who practice molecular pathology and researchers investigating molecular mechanisms of disease, pathology residents and fellows in molecular genetic pathology training programs, and clinicians and researchers interested in advances in molecular diagnostics.

Educational Objectives

At the completion of the 2015 *JMD* CME Program in Molecular Diagnostics, participants should be able to:

1. discuss the research underway and/or current molecular approaches to the diagnosis and prognosis of inherited diseases and syndromes;
2. discuss the research underway and/or current molecular approaches to pharmacogenetics, cytogenetics, DNA identity tests, and hematopathology (including clonality, translocations, and point mutations);
3. discuss the research underway and/or current molecular approaches to the diagnosis and prognosis of solid and soft tissue tumors;
4. discuss the research underway and/or current molecular approaches to the diagnosis of infectious diseases (including bacterial, fungal, viral, and parasitic pathogens);
5. discuss the research underway and/or current molecular approaches to the diagnosis and prognosis of acquired diseases spanning systems biology;
6. demonstrate a gained level of knowledge of the molecular methods and techniques being used by researchers and practitioners;

Disclosure of Financial Relationships and Resolution of Conflicts of Interest

In order to ensure balance, independence, objectivity and scientific rigor in all its educational activities, and in accordance with ACCME Standards, the ASCP requires that all individuals in a position to influence and/or control the content of ASCP CME activities disclose to the ASCP and subsequently to learners whether they do or do not have any relevant financial relationships with proprietary entities producing health care goods or services that are discussed in CME activities.

Faculty are asked to use generic names in any discussion of therapeutic options, to base patient care recommendations on scientific evidence and to base information regarding commercial products/services on scientific methods generally accepted by the medical community. All ASCP CME activities are evaluated by participants for the presence of any commercial bias and thus input is used for subsequent CME planning decisions. The primary purpose of this journal-based CME activity is educational and the comments, opinions, and/or recommendations expressed by the faculty or authors are their own and not those of ASCP or ASIP.

The planning committee members and staff of this journal-based CME activity have no relevant financial relationships with commercial interest to disclose. Relevant financial relationships of the authors of selected articles in this journal-based CME activity will be disclosed on the published article and in each examination.

ASIP 2015 Journal CME Programs

JMD 2015 CME Program in Molecular Diagnostics

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

The Journal of Molecular Diagnostics, Volume 17, Number 1 (January 2015)

www.asip.org/CME/journalCME.htm

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

ANSWERS for CME January Questions # 1-8
1d, 2c, 3d, 4b, 5a, 6d, 7c, 8a

Three articles (on molecular profiling of aggressive B-cell lymphomas, DNA minimal residual disease (MRD) markers in neuroblastoma, and a high-resolution melting (HRM) curve screening test for *SRSF2* mutations in myelodysplastic syndromes) were selected for the **January 2015 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: Carey CD, Gusenleitner D, Chapuy B, Kovach AE, Kluk MJ, Sun HH, Crossland RE, Bacon CM, Rand V, Cin PD, Le LP, Neuberg D, Sohani AR, Shipp MA, Monti S, Rodig SJ: Molecular classification of MYC-driven B-cell lymphomas by targeted gene expression profiling of fixed biopsy specimens. J Mol Diagn 2015, 17:19-30; http://dx.doi.org/10.1016/j.jmoldx.2014.08.006

Questions #4-5 are based on: van Wezel EM, Zwijnenburg D, Zappeij-Kannegieter L, Bus E, van Noesel MM, Molenaar JJ, Versteeg R, Fiocco M, Caron HN, van der Schoot CE, Koster J, Tytgat GAM: Whole-genome sequencing identifies patient-specific DNA minimal residual disease markers in neuroblastoma. J Mol Diagn 2015, 17:43-52; http://dx.doi.org/10.1016/j.jmoldx.2014.09.005

Questions #6-8 are based on: Garza E, Fabiani E, Noguera N, Panetta P, Piredda ML, Borgia L, Maurillo L, Catalano G, Voso MT, Lo-Coco F: Development of a high-resolution melting curve analysis screening test for *SRSF2* splicing factor gene mutations in myelodysplastic syndromes. J Mol Diagn 2015, 17:85-89; http://dx.doi.org/10.1016/j.jmoldx.2014.08.002

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

- Define Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL).
- Understand the genetics of BL and DLBCL.
- Describe B-cell lymphoma unclassifiable (BCL-U).
- Understand the characteristics of neuroblastoma.
- Describe qPCR-based minimal residual disease (MRD) detection.
- Define *SRSF2.*
- Describe the frequency of *SRSF2* mutations in different disorders.
- Define the *SRSF2* hotspot.

1. Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) are aggressive tumors of mature B cells that are distinguished by a combination of histomorphologic, phenotypic, and genetic features. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:19-30.]

a. The World Health Organization (WHO) classification of tumors defines neoplastic diseases according to unique clinical and biological characteristics.
b. The reliable differentiation of BL from DLBCL is important, because these tumors are treated with distinct chemotherapeutic regimens.
c. BL is a neoplasm composed of monomorphic, intermediate-sized lymphocytes that are positive for markers of mature, germinal-center B cells and negative for the anti-apoptotic protein BCL2.
d. Some BL cells (<60%) are positive for the proliferation marker Ki-67/MIB1.

Rationale: Most BL cells (>95%) are positive for the proliferation marker Ki-67/MIB1.

2. BL and DLBCL are aggressive tumors of mature B cells categorized as individual tumor types. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:19-30.]

a. The genetic hallmark of BL is a balanced translocation involving the *MYC* oncogene and the immunoglobulin heavy chain locus (*IGH*).
b. Mutations in *TCF3* and *ID3* are common in BL.
c. DLBCL is composed of pleomorphic, small lymphoid cells and, in general, increased apoptosis and a higher proliferation index than BL.
d. DLBCLs express markers of mature B cells, with or without evidence of germinal center cell derivation, and often express BCL2.

Rationale: DLBCL is composed of pleomorphic, large lymphoid cells and, in general, less apoptosis and a lower proliferation index than BL.

3. A subset of B-cell lymphomas has one or more characteristics that overlap BL and DLBCL, and are categorized as B-cell lymphoma unclassifiable (BCL-U). Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:19-30.]

a. Most cases of BL and DLBCL are diagnosed with high confidence using traditional histopathological, immunophenotypic, and targeted genetic analysis.
b. The 2008 WHO Classification of Lymphoid Tumors recognized cases with the novel diagnostic category, BCL-U, as having features intermediate between DLBCL and BL.
c. BCL-U is, by definition, a heterogeneous group, and its diagnosis requires that pathologists make subtle distinctions in histomorphological features, immunophenotype, and genetics that may not be highly reproducible.
d. High co-expression of MYC and TCF3 in tumor cells provides a biological basis for the inferior outcome among patients with the activated B-cell (ABC) type BL when treated with standard chemotherapy.

Rationale: High co-expression of MYC and BCL2 in tumor cells provides a biological basis for the inferior outcome among patients with the ABC type DLBCL when treated with standard chemotherapy.

4. Neuroblastoma is an extracranial solid tumor of childhood, with a broad spectrum of clinical behavior. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:43-52.]

a. Despite intensive treatment, high-risk neuroblastoma patients have a poor prognosis, and relapse is a frequent occurrence.
b. Bone marrow (BM) metastases are present in approximately 80% of patients at diagnosis.
c. Quantitative real-time PCR (qPCR) is a sensitive technique to detect small numbers of tumor cells in blood or BM.
d. qPCR-based minimal residual disease (MRD) detection is based on neuroblastoma-specific RNA markers.

Rationale: Bone marrow (BM) metastases are present in approximately 50% of patients at diagnosis.

5. RNA MRD markers can have disadvantages. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:43-52.]

a. The MRD level might be overestimated by using RNA markers.
b. The expression of RNA markers can vary between patients.
c. It is unknown whether RNA markers are stably expressed during treatment.
d. Only paired-like homeobox 2b (PHOX2B) has no expression in hematologic cells.

Rationale: The MRD level might be underestimated by using RNA markers. Overall, the MRD levels determined by DNA markers were comparable to RNA-based PCRs in 16 of 22 samples. In 6 samples even a higher MRD level was found for the DNA markers. The optimal method for MRD detection will have to be investigated by comparing the sensitivity and clinical utility of DNA and RNA markers in paired samples of a large cohort of patients.

6. Somatic mutations of the spliceosome machinery have been identified using whole genome analysis in several hematologic diseases and solid tumors. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17: 85-89.]

a. *SRSF2* is a member of the serine/arginine-rich family pre-mRNA splicing factors that is involved in mRNA export from the nucleus and translation.
b. Besides the RNA recognition domain, the SRSF2 protein contains a serine/arginine-rich domain that promotes interaction with other splicing factors.
c. SRSF2 constitutes a critical player in the process of mRNA splicing.
d. The *SRSF2* gene is located on the short arm of chromosome 9 subregion 14.2.

Rationale: The *SRSF2* gene is located on the short arm of chromosome 17 subregion 25.1.

7. *SRSF2* mutations have been described in several hematologic disorders. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17: 85-89.]

a. In one study, *SRSF2* mutations were detected in 5.5% of refractory anemia with ringed sideroblasts and refractory cytopenia with multilineage dysplasia with ringed sideroblasts.
b. *SRSF2* mutations were detected in 28.4% of chronic myelomonocytic leukemias (CMMLs) in one report, whereas another report identified the mutations in nearly half of the cases.
c. A total of 13.3% of *SRSF2* mutations have been found in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS).
d. *SRSF2* mutations have been detected in 0.7% *de novo* AML and in 1.9% of myeloproliferative neoplasms.

Rationale: In one study, *SRSF2* mutations in hematologic disorders were reported to occur at a frequency of 6.5% in AML and MDS, 11.6% in MDS without ringed sideroblasts, 5.5% in refractory anemia with ringed sideroblasts and refractory cytopenia with multilineage dysplasia with ringed sideroblasts, 0.7% in *de novo* AML, 1.9% in myeloproliferative neoplasms, and 28.4% in CMML. Another study reported that *SRSF2* mutations were present in 47% of a cohort of 275 CMML patients.

8. Most *SRSF2* mutations are located at the amino acid position P95. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17: 85-89.]

a. The hotspot localization at P95 corresponds to exon 4 of the *SRSF2* gene.
b. Most P95 mutations are missense in which proline is substituted by histidine (47.5%), leucine (31.6%), arginine (19.2%), or rarely, alanine or threonine.
c. In primary myelofibrosis, *SRSF2* monoallelic mutations were reported in 32 of 187 patients, affecting residue P95.
d. *SRSF2* mutations are significantly associated with advanced age, high-risk category, and worse prognostic outcome compared with nonmutated primary myelofibrosis.

Rationale: The hotspot localization at P95 corresponds to exon 1 of the *SRSF2* gene.

ASIP 2015 Journal CME Programs

JMD 2015 CME Program in Molecular Diagnostics

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

The Journal of Molecular Diagnostics, Volume 17, Number 2 (March 2015)

www.asip.org/CME/journalCME.htm

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

ANSWERS for CME March Questions # 1-8
1b, 2c, 3b, 4d, 5c, 6a, 7c, 8d

Articles on a screening assay for myotonic dystrophy type 1 (DM1) and on miR-210 as a prognostic marker in clear cell renal cell carcinoma were selected for the **March 2015 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: Lian M, Rajan-Babu I, Singh K, Lee CG, Law H, Chong SS: Efficient and highly sensitive screen for myotonic dystrophy type 1 using a one-step triplet-primed PCR and melting curve assay. J Mol Diagn 2015, 17:128-135; http://dx.doi.org/10.1016/j.jmoldx.2014.10.001

Questions #5-8 are based on: Samaan S, Khella HWZ, Girgis A, Scorilas A, Lianidou E, Gabril M, Krylov SN, Jewett M, Bjarnason GA, El-said H, Yousef GM: miR-210 is a prognostic marker in clear cell renal cell carcinoma. J Mol Diagn 2015, 17:136-144; http://dx.doi.org/10.1016/j.jmoldx.2014.10.005

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

- Understand the causes of myotonic dystrophy type 1 (DM1).
- Define the expansion of trinuceotide repeats in normal and premutation alleles of the dystrophia myotonica-protein kinase (*DMPK*) gene.
- Describe the available assays for the molecular detection of the *DMPK* CTG repeat expansion.
- Describe the triplet-primed PCR molecular testing strategy.
- Define renal cell carcinoma (RCC).
- Describe the 5-year survival rate of clear cell RCC (ccRCC).
- Understand the prognostic assessment of RCC.
- Describe miRNAs and their involvement in RCC pathogenesis.

1. Myotonic dystrophy type 1 (DM1) is an autosomal dominant neuromuscular disorder with a broad spectrum of clinical features. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17: 128-135.]

a. DM1 is caused by abnormal expansion of a CTG repeat in the 3' untranslated region of the dystrophia myotonica-protein kinase (*DMPK*) gene on chromosome 19q13.3.
b. Myotonic dystrophy type 2 (DM2) is also caused by mutations in the *DMPK* gene.
c. Among affected individuals, size of the expanded CTG repeat positively correlates with phenotypic severity and negatively correlates with age of disease onset.
d. DM1 demonstrates a global prevalence of 1 in 8000.

Rationale: DM1 and DM2 are genetically distinct disorders. (DM2 is caused by a defect in the *CNBP* gene on chromosome 3 and is a tetranucleotide repeat disorder.) DM1 also shares overlapping clinical features with other nondystrophic myotonias, all of which are genetically distinct disorders. Therefore, molecular detection of the *DMPK* CTG repeat expansion is essential for definitive diagnosis of DM1.

2. DM1 is the most common adult-onset neuromuscular disorder. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17: 128-135.]

a. Normal *DMPK* alleles are highly polymorphic in the general population, with repeat sizes ranging from 5 to 34 CTGs that are stably transmitted across generations.
b. Individuals with *DMPK* premutation alleles of 35 to 49 repeats are asymptomatic but are at an increased risk of transmitting alleles of larger repeat sizes to their offspring.
c. Among DM1 patients carrying mutant *DMPK* alleles, disease phenotypes are grouped into three categories based on CTG repeat size: mild DM1 (35 to 99 repeats), classic DM1 (100 to 1000 repeats), and congenital DM1 (>1000 repeats).
d. CTG repeat size information is potentially useful in predicting disease severity and age at onset; however, there appears to be no significant correlation between size and age at onset beyond a threshold repeat size, in particular for classic DM1.

Rationale: Disease phenotypes are grouped into three categories based on CTG repeat size: mild DM1 (50 to 150 repeats), classic DM1 (100 to 1000 repeats), and congenital DM1 (>1000 repeats).

3. Molecular detection of the *DMPK* CTG repeat expansion is essential for definitive diagnosis of DM1. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17: 128-135.]

a. Early molecular methods used repeat-spanning PCR, which detected and sized only normal-to-small expansion alleles.
b. Southern blot analysis, although labor intensive, is the preferred method for detecting small to medium expansions.
c. Triplet-primed PCR (TP-PCR) uses characteristic differences in amplicon pattern between normal and expanded alleles to differentiate between them.
d. TP-PCR effectively detects all *DMPK* alleles.

Rationale: Southern blot analysis excels at detecting large expansions but is labor intensive, time-consuming, and requires microgram amounts of DNA. Previously, upon identification of a homozygous sample by repeat-spanning PCR, Southern blot analysis was required to rule out large CTG repeat expansions. The increasing adoption of TP-PCR, with its ability to detect expansion mutations regardless of size, has reduced the need to perform Southern blot analysis. TP-PCR is the preferred method for detecting all *DMPK* alleles.

4. The TP-PCR strategy is widely preferred for the molecular testing of DM1. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:128-135.]

a. TP-PCR is recommended by the European Molecular Genetics Quality Network for samples showing apparent homozygosity for a normal allele after repeat-spanning PCR.
b. The discovery of interruptions within the CTG repeat raised the possibility of TP-PCR failure due to inability of the triplet-primed primer to anneal.
c. Bidirectional TP-PCR targeting both the 5' and 3' ends of the CTG repeat is used to prevent false-negative interpretations caused by interruptions in expanded alleles.
d. For large-scale screening purposes, capillary electrophoresis is a cost-effective downstream analysis procedure after TP-PCR.

Rationale: Capillary electrophoresis is currently the preferred downstream analysis procedure after TP-PCR, but is not cost effective for large-scale screening purposes. The authors describe a cost-effective single-step screening procedure for rapid detection of *DMPK* repeat expansions based on automated melting curve analysis (MCA) of bidirectional TP-PCR products.

5. Epidemiologic data have shown a rapid rise in the incidence of renal cell carcinoma (RCC). Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17: 136-144.]

a. RCC is the most common type of kidney cancer in adults.
b. RCC encompasses a number of cancer subtypes that have distinct structural and cytogenetic characteristics, including clear cell (cc-RCC), papillary, and chromophobe subtypes.
c. The most common RCC subtype is papillary, accounting for 50% of RCC cases.
d. The morphologic classification is not always accurate and in the same subtype subgroups have shown distinct biological behavior.

Rationale: The most common RCC subtype is ccRCC, accounting for 80% of RCC cases.

6. The 5-year survival rate varies greatly in ccRCC. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17: 136-144.]

a. The 5-year survival of ccRCC ranges from 80% to 85% for tumors <4 cm, to approximately 40% in locally aggressive tumors that extend through the renal capsule or to the renal sinus.
b. The 5-year survival for metastatic tumors drops significantly to approximately 5% to 15%.
c. An accurate assessment of prognosis is essential in guiding the treatment decision for both primary and metastatic kidney cancer.
d. In addition to surgical removal, other options for early-stage cancer include watchful waiting and percutaneous ablation of tumors.

Rationale: The 5-year survival of ccRCC ranges from 90% to 95% for tumors <4 cm, to approximately 60% in locally aggressive tumors that extend through the renal capsule or to the renal sinus.

7. Accurate assessment of prognosis of ccRCC is key in optimizing management plans to fit individual patient needs. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17: 136-144.]

a. The prognostic assessment of RCC currently relies on clinical models.
b. Studies have investigated the prognostic value of clinical parameters such as tumor size and staging of tumor, node, and metastasis (TNM), in ccRCC.
c. The T1 stage of ccRCC is subdivided into three groups according to TNM.
d. Small renal masses can be classified as either progressive or nonprogressive according to their biological behavior.

Rationale: The T1 stage of ccRCC is subdivided into two groups according to tumor size.

8. miRNAs are short noncoding RNA nucleotides that regulate target expression post-transcriptionally. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:136-144.]

a. miRNAs are dysregulated in RCC, pointing to their involvement in RCC pathogenesis.
b. miRNAs have the potential to be useful diagnostic and prognostic markers as well as therapeutic targets.
c. miRNAs are documented to be downstream effector molecules of the hypoxia inducible factor (HIF)-induced hypoxia response and may be involved in non-HIF–mediated pathways .
d. miR-160 has been implicated as a clinical marker because of its involvement with hypoxia in various cancers, including breast, colon, and pancreatic cancers.

Rationale: miR-210 has been implicated as a clinical marker because of its involvement with hypoxia in various cancers, including breast, lung, and pancreatic cancers.

ASIP 2015 Journal CME Programs

JMD 2015 CME Program in Molecular Diagnostics

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

The Journal of Molecular Diagnostics, Volume 17, Number 3 (May 2015)

www.asip.org/CME/journalCME.htm

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

ANSWERS for CME May Questions # 1-8
1b, 2d, 3c, 4d, 5b, 6d, 7d, 8a

A Special Article on molecular genetic test utilization and a Review on the discovery of novel viruses in emerging infectious diseases were selected for the **May 2015 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 #1-2 are based on: Riley JD, Procop GW, Kottke-Marchant K, Wyllie R, Lacbawan FL: Improving molecular genetic test utilization through order restriction, test review, and guidance. J Mol Diagn 2015, 17:225-229; http://dx.doi.org/10.1016/j.jmoldx.2015.01.003

Questions #3-8 are based on: Sridhar S, To KKW, Chan JFW, Lau SKP, Woo PCY, Yuen KY: A systematic approach to novel virus discovery in emerging infectious disease outbreaks. J Mol Diagn 2015, 17:230-241; http://dx.doi.org/10.1016/j.jmoldx.2014.12.002

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

- Give examples of novel viruses and explain how they contribute to human disease.
- Understand the importance of specimen collection.
- Describe viral visualization and when electron microscopy (EM) is utilized.
- Define consensus-degenerate hybrid oligonucleotide primers (CODEHOPs).
- Describe the representational difference analysis (RDA) method and when it is used.
- Define rolling circle amplification (RCA) and what it amplifies.
- Understand the complexities of genetic and genomic testing to laboratory medicine.

1. The ordering of molecular genetic tests by health providers not well trained in genetics may have a variety of untoward effects. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:225-229.]

a. These effects include the selection of inappropriate tests, the ordering of panels when the assessment of individual or fewer genes would be more appropriate, inaccurate result interpretation and inappropriate patient guidance, and significant unwarranted cost expenditure.
b. Genetic and genomic testing is clinically available for >8000 genetic conditions.
c. Genetic and genomic testing, although fairly low volume relative to other laboratory tests, contributes substantial cost to laboratory medicine.
d. The substantial cost to laboratory medicine is in part because of the increasing availability and complexity of molecular test options.

Rationale: Genetic and genomic testing is clinically available for >4000 genetic conditions, a number that has tripled in the past decade.

2. Traditional approaches to improving test utilization are being challenged in the current health care climate. Based on the referenced Special Article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:225-229.]

a. A study of United Healthcare members found that spending on molecular genetic tests increased 34% per year between 2008 and 2010.
b. Given the rarity of most genetic disorders and the growing array of testing options, it is perhaps not surprising that 8% to 30% of genetic tests are ordered incorrectly.
c. Many physicians report feeling unprepared to order genetic testing or perform clinical tasks related to genetics because of lack of knowledge, confidence, and experience with genetic disorders.
d. Difficulties associated with ordering molecular genetic tests almost certainly contribute to delayed time to diagnosis and an increase in the risk of erroneous result interpretation.

Rationale: A study of United Healthcare members found that spending on molecular genetic tests increased 14% per year between 2008 and 2010.

3. Novel viruses are important causes of emerging infectious diseases. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:230-241.]

a. Two novel coronaviruses in recent times have been identified, including severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV).
b. Outside of outbreak settings, many clinical syndromes encountered by clinicians on a daily basis have no identifiable infectious etiology, raising the possibility of infections by as-yet undiscovered pathogens.
c. At the present time, nearly 100 viruses are known to cause human disease.
d. Extrapolation of recent trends anticipates that pathogenic virus discovery is likely to continue unabated in the near future.

Rationale: At the present time, >200 viruses are known to cause human disease; extrapolation of recent trends anticipates that pathogenic virus discovery is likely to continue unabated in the near future.

4. Careful specimen collection from patients suspected to harbor novel viruses is crucial to their successful recovery. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:230-241.]

a. Clinical specimens must be of good quality and obtained serially throughout the course of illness to capture the novel virus at the time of peak viral load.
b. Viral load dynamics vary, but in general, specimens that are collected early in the course of the illness just before the illness nadir are likely to contain a high viral load.
c. The clinical syndrome often dictates the sites of specimen collection.
d. Throat swab specimens are particularly valuable in patients presenting with pneumonia.

Rationale: The clinical syndrome often dictates the sites of specimen collection. For example, lower respiratory tract specimens are particularly valuable in patients presenting with pneumonia.

5. Electron microscopy (EM) offers an unbiased method for visualization of virus-like particles in clinical specimens, cell culture fluid, and tissue sections. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:230-241.]

a. Detection by EM is possible as long as the virus is present in sufficient concentrations in the specimen and is not affected by the viability of the virus.
b. Successful visualization of virus by EM requires a virus concentration of 10^2 to 10^3 particles/mL of specimen.
c. Plasma usually has sufficient concentration of virus to permit EM detection of hepatitis B, ebola, and early phase of parvovirus B19 virus infections, and vesicular fluid usually contains sufficient concentration of poxvirus and herpes viruses.
d. EM is commonly used for visualization of putative novel viruses producing cytopathic effect in culture systems and facilitating choice of consensus primers based on morphological appearance.

Rationale: Successful visualization of virus by EM requires a virus concentration of 10^5 to 10^6 particles/mL of specimen.

6. If the novel virus is strongly suspected to be a member of a known group of viruses, then pools of degenerate primers that encompass all possible nucleotide differences in a given sequence may be used to amplify gene segments. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:230-241.]

a. Degenerate primers are derived from amino acid motifs of highly conserved proteins of the virus group of interest.
b. Consensus primers are derived directly from the nucleotide sequences of conserved proteins of a particular virus family.
c. A different strategy combining the strengths of degenerate and consensus primers is the usage of consensus-degenerate hybrid oligonucleotide primers (CODEHOPs).
d. CODEHOPs contain a degenerate 5' core region and a conserved 3' clamp sequence.

Rationale: CODEHOPs contain a degenerate 3' core region and a conserved 5' clamp sequence. The limited level of degeneracy in the 3' core maintains the relative lack of bias of degenerate primers. The conserved 5' clamp sequence stabilizes hybridization of the 3' core and allows higher annealing temperatures.

7. Representational difference analysis (RDA) was first developed for defining the difference between tester DNA, believed to contain the target nucleic acid, and driver DNA, which represents the wild-type. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:230-241.]

a. Oligonucleotide adaptor ligation and adaptor-specific PCR amplifies both cDNA populations (tester and wild-type).
b. Hybridization of tester with adaptor-ligated driver DNA follows the ligation and PCR steps.
c. Sequences common to both tester and wild-type populations are subtracted while adaptor-specific primers amplify sequences unique to the tester population for subsequent sequencing.
d. The discovery of polyomavirus was made using the RDA method.

Rationale: The RDA method was used to discover human herpesvirus 8 and TT virus. Polyomavirus sequences in patients with Merkel cell carcinoma were discovered using digital transcriptome subtraction.

8. Rolling circle amplification (RCA) enables detection of novel viruses that possess a circular DNA genome. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:230-241.]

a. RCA has been used for the discovery of circular DNA viruses such as herpesviruses.
b. RCA protocols for the detection of novel viruses use multiple random hexamer primers that bind to different points of the circular genome.
c. Most RCA applications use phi29 DNA polymerase, which possesses strand displacement and 3' to 5' exonuclease activities to produce linear concatamerized double stranded DNA copies of the novel viral genome.
d. After the phi29 DNA polymerase step, PCR products are digested with a restriction enzyme that is likely to only have a single recognition site in the viral genome. Gel electrophoresis, sequencing, and phylogenetic analysis complete the process.

Rationale: Herpesviruses contain linear DNA. RCA has been used for the discovery of several novel circular DNA viruses such as papillomaviruses, polyomaviruses, and anneloviruses.

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

ANSWERS for CME July Questions # 1-8
1b, 2a, 3c, 4d, 5d, 6a, 7b, 8a

A Review on annotating somatic variants in cancer and an article on the evaluation of mutational testing of pre-neoplastic Barrett's mucosa were selected for the **July 2015 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 #1-6 are based on: Lee LA, Arvai KJ, Jones D: Annotation of sequence variants in cancer samples: Processes and pitfalls for routine assays in the clinical laboratory. J Mol Diagn 2015, 17:339-351; http://dx.doi.org/10.1016/j.jmoldx.2015.03.003. Please note that at the time the Review was composed, all the authors were employees of Quest Diagnostics, which offers sequencing assays commercially.

Questions #7-8 are based on: Del Portillo A, Lagana SM, Yao Y, Uehara T, Jhala N, Ganguly T, Nagy P, Gutierrez J, Luna A, Abrams J, Liu Y, Brand R, Sepulveda JL, Falk GW, Sepulveda AR: Evaluation of mutational testing of preneoplastic Barrett's mucosa by next-generation sequencing of formalin-fixed, paraffin-embedded endoscopic samples for detection of concurrent dysplasia and adenocarcinoma in Barrett's esophagus. J Mol Diagn 2015, 17:412-419; http://dx.doi.org/10.1016/j.jmoldx.2015.02.006

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

- Describe the genetic changes that are associated with clinical syndromes such as cancer.
- Define minor allele frequencies (MAFs).
- Describe driver and passenger mutations and their role in tumor development.
- Explain next-generation sequencing (NGS) assays and their use.
- Define *TET2* and the mutations that occur in this gene.
- Describe pathway analysis and co-occurring mutations.
- Describe the characteristics of Barrett's esophagus.
- Understand the risk factors, prevention, and detection of esophageal adenocarcinoma (EAC).

1. During tumor development, there is a complex interplay between somatic or acquired mutations in oncogenes, tumor suppressors, and epigenetic regulators and germline, or inherited, genetic variation. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:339-351.]

a. Initial work on localizing genetic variants associated with cancer susceptibility focused on well-defined clinical syndromes.
b. Oncogenes were initially localized using targeted DNA sequencing.
c. Genetic changes observed in affected individuals include frameshift and nonsense mutations.
d. Additional genetic changes involve inactivating mutations with loss of function linked to tumor initiation.

Rationale: Initial work on localizing genetic variants associated with cancer susceptibility focused on tumor suppressor genes, which were initially localized using linkage analysis and targeted DNA sequencing.

2. Translation of germline variant calls into clinical decisions relies on proper annotation. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:339-351.]

a. Presumed benign variants are typically regarded as those with minor allele frequencies (MAFs) of 10% to 15%.
b. Most single nucleotide polymorphisms (SNPs) occur at MAFs under 0.5% (<1% of the population), highlighting the difficulty of variant annotation.
c. At this time, curated gene-specific databases cover only a few cancer-associated genes.
d. The best-studied cancer susceptibility genes, particularly *BRCA1*, *BRCA2*, and the Lynch syndrome-associated mismatch repair genes, have publicly available and curated databases.

Rationale: Presumed benign variants are typically regarded as those with MAFs >1% to 5%.

3. Genetic changes that arise during the development of a tumor are termed somatic mutations and possess commonalities and differences with germline changes. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:339-351.]

a. Acquired somatic mutations in cancer cells are propagated through clonal expansion from founder cancer stem cells or tumor subpopulations.
b. If a given genetic change promotes tumor development, it is regarded as a driver mutation and is typically retained during the disease course.
c. Driver mutations are typically classified as loss-of-function changes in tumor-promoting oncogenes or gain-of-function changes in tumor suppressor genes.
d. Once a tumor becomes established, additional mutations that were present in the selected abnormal cell population but that are not integral to tumorigenesis can arise as passengers.

Rationale: Driver mutations are typically classified as gain-of-function changes in tumor-promoting oncogenes or loss-of-function changes in tumor suppressor genes.

4. Approaches to somatic variant annotation in cancers differ based on type of assay (full exome versus hotspot or targeted panels) and assay goal(s). Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:339-351.]

a. Next-generation sequencing (NGS) clonality assays include T-cell receptor and B-cell antigen receptor profiling in lymphoproliferative disorders, identifying hematologic neoplasms in patients with blood cell abnormalities and distinguishing atypical hyperplasia from early-stage precancers lesions.
b. If a sequencing assay is used for risk stratification, annotation must be tied to an underlying data set with strong statistical power and a similar clinical management strategy as the target population.
c. For theranostic indications, annotation focuses on identifying important driver or resistance mutations that can guide therapy decisions.
d. NGS theranostic panels comprising thousands of genes are now routinely used, particularly for relapsed or refractory solid tumors for which off-label or compassionate use of targeted agents is more common.

Rationale: NGS theranostic panels comprising tens to hundreds of genes are now routinely used, particularly for relapsed or refractory solid tumors for which off-label or compassionate use of targeted agents is more common.

5. Mutations in *TET2* are now commonly used to help in the diagnosis and classification of myelodysplastic syndrome and myeloproliferative neoplasm. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:339-351.]

a. *TET2* belongs to the TET family of epigenetic regulatory enzymes that convert 5-methyl-cytosine to 5-hydroxymethylcytosine.
b. Somatic or acquired *TET2* mutations occur at high frequency across a spectrum of myeloid and lymphoid malignant tumors.
c. Most pathogenic mutations in *TET2* result in complete or partial loss of function.
d. *TET2* mutations are commonly missense mutations occurring anywhere within the coding region of the gene.

Rationale: Most pathogenic mutations in *TET2* result in complete or partial loss of function, evidenced by attenuation of 5-hydroxymethyl-cytosine in leukocyte DNA from patients with *TET2*-mutated blood cells compared with those in unaffected populations. These mutations are commonly frameshift and nonsense or terminating mutations occurring anywhere within its 2002 amino acids but also include many different missense mutations that are presumed to have hypofunctional effects.

6. Pathway analysis is a tool in which genes that are complementary are frequently mutated together, whereas those that act along the same pathway or in the same complex are not. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:339-351.]

a. In hematopoietic malignant tumors, *TET2* mutations typically co-occur with mutations in the epigenetic regulators *DNMT3B* and *DNMT1*.
b. *TET2* mutations are mutually exclusive with *IDH1* and *IDH2* mutations in hematopoietic malignant tumors.
c. The frequency of complementing mutations increases with tumors progression.
d. As knowledge of mutational patterns characteristic for specific cancer types increases, the pattern of co-occurring alterations in other genes can help resolve the nature of indeterminate calls.

Rationale: In hematopoietic malignant tumors, *TET2* mutations typically co-occur with mutations in the epigenetic regulators *DNMT3A* and *EZH2* but are mutually exclusive with *IDH1* and *IDH2* mutations.

7. Esophageal adenocarcinoma (EAC) most frequently develops in patients with Barrett's esophagus (BE), estimated to affect 3.3 million adults in the United States. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:412-419.]

a. BE results from injury of the esophageal mucosa associated with gastroesophageal reflux, which leads to esophagitis and eventually BE.
b. The incidence of EAC has doubled over the past four decades in the United States, paralleling the increase in detection of esophageal reflux and diagnosis of BE.
c. Barrett's intestinal metaplasia (BIM) is characterized by the replacement of normal squamous esophageal mucosa by columnar epithelium with intestinal metaplasia.
d. BIM often occurs in the background of patches of cardiac, oxyntic, or cardio-oxyntic type mucosa along the length of BE.

Rationale: The incidence of EAC has increased greater than fivefold over the past four decades in the United States, paralleling the increase in detection of esophageal reflux and diagnosis of BE.

8. BE may harbor genomic mutations before histologic appearance of dysplasia and cancer and requires frequent surveillance. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:412-419.]

a. Patients with BE without dysplasia have the same EAC risk as those with high-grade dysplasia.
b. Current guidelines for prevention of EAC require repeat surveillance endoscopies with biopsies of the Barrett's mucosa, followed by pathological examination to detect Barrett's intestinal metaplasia and dysplasia.
c. The detection of dysplasia is hampered by sampling errors and high interobserver diagnostic variability.
d. Known risk factors associated with EAC include male sex, older age, white race, hiatal hernia size, length of Barrett's epithelium, smoking, and high body mass index.

Rationale: Patients with BE without dysplasia have a lower EAC risk (0.1% to 0.5% per patient-year) than those with high-grade dysplasia (6% to 19% per patient-year).

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ANSWERS for CME September Questions # 1-8
1b, 2c, 3a, 4c, 5b, 6d, 7b, 8a

A Review article on next-generation sequencing for hereditary cancer syndromes and an article on detection of paternally inherited mutations in maternal plasma were selected for the **September 2015 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 #1-6 are based on: Tafe LJ: Targeted next-generation sequencing for hereditary cancer syndromes: A focus on Lynch syndrome and associated endometrial cancer. J Mol Diagn 2015, 17:472-482; http://dx.doi.org/10.1016/j.jmoldx.2015.06.001

Questions #7-8 are based on: van den Oever JME, van Minderhout IJHM, Harteveld CL, den Hollander NS, Bakker E, van der Stoep N, Boon EMJ: A novel targeted approach for noninvasive detection of paternally inherited mutations in maternal plasma. J Mol Diagn 2015, 17:590-596; http://dx.doi.org/10.1016/j.jmoldx.2015.05.006

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

- Describe Lynch syndrome (LS) and the genes associated with its development.
- Understand the association between LS and endometrial cancer (EC).
- Define screening procedures for LS.
- Understand immunohistochemical studies used for LS screening.
- Describe microsatellite instability (MSI) testing on colorectal cancer tissue.
- Explain the different approaches to next-generation sequencing (NGS) testing for cancer.
- Understand the application of noninvasive prenatal diagnostics (NIPD) testing.
- Describe high-resolution melting curve analysis (HR-MCA).
- Understand how locked nucleic acid (LNA) probes can affect specificity and sensitivity of mutation testing.

1. Lynch syndrome (LS) is a hereditary cancer syndrome that results from germline mutations in one of the DNA mismatch repair genes. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:472-482.]

a. The mismatch repair system (MMR) proteins form heterodimers, which excise and coordinate repair of single base pair mismatches and small loops or bubbles in DNA that can arise during replication due to slippage of DNA polymerase.
b. LS is associated with an increased lifetime risk of colorectal cancer (CRC) (20% to 42% in men; 54% to 74% in women), and endometrial cancer (EC) (9% to 12%) and ovarian cancer (20% to 60%) in women.
c. To a lesser extent, LS is associated with an increased lifetime risk of gastric, urinary tract, small bowel, pancreatobiliary tract, and brain cancers.
d. Current testing algorithms include a detailed patient history, screening CRC or EC for defects in MMR protein expression by immunohistochemistry (IHC), microsatellite instability (MSI) testing, *BRAF* testing (for CRC), and, when appropriate, *MLH1* promoter methylation analysis.

Rationale: LS is associated with an increased lifetime risk of CRC (54% to 74% in men; 30% to 52% in women), EC (20% to 60% in women), and ovarian cancer (9% to 12% in women) as well as gastric, urinary tract, small bowel, pancreatobiliary tract, and brain cancers to a lesser extent.

2. EC is an often overlooked association with LS. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:472-482.]

a. EC develops in 20% to 60% of women with LS.
b. Approximately 50% of women with LS are predicted to have EC as their sentinel diagnosis and up to 14% to have synchronous cancers at diagnosis.
c. The mutation frequency of MMR genes in EC is 50% to 66% for *MLH1*, 24% to 40% for *MSH2*, 10% to 13% for *PMS2*, and <5% for *MSH6*.
d. Defects in the MMR system lead to errors in DNA replication, particularly in microsatellites, or areas of short tandem repetitive DNA sequences.

Rationale: The mutation frequency of MMR genes in EC is 24% to 40% for *MLH1*, 50% to 66% for *MSH2*, 10% to 13% for *MSH6*, and <5% for *PMS2*.

3. Current testing algorithms for LS include a patient history and laboratory-based tests. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:472-482.]

a. Bethesda guidelines recommend MSI testing for individuals with CRC diagnosed in a patient >60 years of age.
b. A substantial number (60% to 65%) of patients with LS-associated EC are >50 years of age at presentation and 60% to 70% do not have a personal family history of LS-associated cancers.
c. It is now recognized that the Amsterdam and Bethesda guidelines, based heavily on a patient's personal and family history, are not sensitive enough to select all individuals at risk of LS for testing.
d. Several organizational guidelines recommend that all CRCs should be screened for LS at the time of diagnosis either by MMR protein IHC or by MSI testing.

Rationale: Currently the revised Bethesda guidelines are the most commonly used criteria to guide molecular studies. These guidelines recommend MSI testing for individuals with CRC diagnosed in a patient <50 years of age.

4. IHC is routinely used for LS screening in clinical laboratories. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:472-482.]

a. When interpreting the IHC studies for expression of the MMR proteins, complete loss of expression in the setting of positive internal control cells is considered a positive result.
b. Because the MMR proteins form heterodimers, the IHC expression pattern can be used to identify the MMR gene that is most likely to be affected by a germline mutation, directing gene-specific testing.
c. Loss of MLH1 expression is almost always accompanied by MSH2 loss, whereas loss of PMS2 expression is almost always accompanied by MSH6 loss.
d. In approximately 70% of the cases, loss of MLH1 expression is because of promoter methylation; methylation-specific PCR-based assays are often used to confirm methylation status.

Rationale: Because the MMR proteins form heterodimers, the IHC expression pattern can be used to identify the MMR gene that is most likely to be affected by a germline mutation, directing gene-specific testing. For example, loss of MLH1 expression is almost always accompanied by PMS2 loss, whereas loss of MSH2 expression is accompanied by MSH6 loss.

5. MSI can routinely be performed on tumor tissue but also requires normal control tissue or blood. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:472-482.]

a. A commercially available panel that includes five mononucleotide markers and two pentanucleotide markers is used by many laboratories to test for MSI; other panels are available, including the National Cancer Institute panel.
b. Instability in four or more of the five mononucleotide markers is classified as MSI-high, whereas instability in two of the five markers is considered MSI-low, and instability in one of the markers is considered MSI stable.
c. The pentanucleotide markers are relatively resistant to instability and are included in the assay to confirm that the tumor and normal control samples are from the same patient.
d. In CRC up to 15% of tumors have MSI and approximately 20% of EC have MSI; the majority of these cases (>70%) have sporadic methylation of the *MLH1* promoter, leading to gene silencing.

Rationale: Instability in two or more of the five mononucleotide markers is classified as MSI-high, whereas instability in only one of the five markers is considered MSI-low. Instability in none of the markers is considered MSI stable.

6. Laboratories have different approaches to next-generation sequencing (NGS) testing for cancer risk predictions. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:472-482.]

a. Approaches include the type of testing used, the number of panels offered, and the number and/or specific genes included on each panel.
b. Several of the commercial and academic reference laboratories currently offer a range of 1 to 10 NGS gene panels that each include from 5 to 43 genes for hereditary cancers.
c. With NGS, false negatives may occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality.
d. It is deemed sufficient to have an average depth of coverage of 150 to 200 times to detect most germline single nucleotide variants.

Rationale: It is deemed sufficient to have an average depth of coverage of 50 to 100 times to detect most germline single nucleotide variants.

7. Since the successful introduction of noninvasive prenatal testing for fetal trisomy screening, requests to expand the repertoire for noninvasive prenatal diagnostics (NIPD) have been increasing. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:590-596.]

a. NIPD can be performed on small fragments of cell-free fetal DNA (cffDNA) that are present in maternal plasma.
b. The amount of cffDNA is insufficient for noninvasive detection in maternal plasma prior to 15 weeks of gestation.
c. Current clinical application of NIPD includes fetal sex determination, fetal rhesus D determination, and the diagnosis of several monogenic disorders.
d. For the diagnosis of monogenic disorders, NIPD can be applied in both autosomal dominant and recessive cases, most efficiently when the mother does not carry the mutant allele and/or carries a different mutation compared with the father, respectively.

Rationale: The amount of cffDNA is sufficient for noninvasive detection in maternal plasma by 7 to 9 weeks of gestation.

8. High-resolution melting curve analysis (HR-MCA) is a relatively simple, fast, and low-cost technique for genotyping and mutation scanning that is frequently used in routine molecular and cancer diagnostics. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:590-596.]

a. HR-MCA combines (asymmetric) PCR with a short pre-PCR melting step to detect sequence variations using a saturating single-stranded DNA binding dye.
b. Although HR-MCA is a relatively sensitive technique, the detection of mosaic or low-level mutations may still be challenging and variant dependent.
c. Locked nucleic acid (LNA) is a bicyclic high-affinity nucleic acid analog that contains a ribonucleoside link between the 2'-oxygen and the 4'-carbon atoms with a methylene unit (2'-O, 4'-C-methylene bridge).
d. In case of mismatch, the LNA probe does not bind to the template with high affinity, enabling primer extension and preferential amplification of the allele of interest.

Rationale: HR-MCA combines (asymmetric) PCR with a short post-PCR melting step to detect sequence variations using a saturating double-stranded DNA binding dye.

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

ANSWERS for CME November Questions # 1-8
1d, 2b, 3a, 4c, 5b, 6d, 7a, 8d

A Special article on next-generation sequencing for infectious disease diagnosis and management, and an article on the utility of *SERPINA1* sequencing for evaluation of patients being screened for genetic α-1 antitrypsin (AAT) deficiency were selected for the **November 2015 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 #1-6 are based on: Lefterova MI, Suarez CJ, Banaei N, Pinsky BA: Next-generation sequencing for infectious disease diagnosis and management: A report of the Association for Molecular Pathology. J Mol Diagn 2015, 17:623-634; http://dx.doi.org/10.1016/j.jmoldx.2015.07.004

Questions #7-8 are based on: Graham RP, Dina MA, Howe SC, Butz ML, Willkomm KS, Murray DL, Snyder MR, Rumilla KM, Halling KC, Highsmith Jr. WE: *SERPINA1* full-gene sequencing identifies rare mutations not detected in targeted mutation analysis. J Mol Diagn 2015, 17:689-694; http://dx.doi.org/10.1016/j.jmoldx.2015.07.002

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

- Describe next-generation sequencing (NGS).
- Contrast the roles of the Sanger sequencing method and NGS in infectious disease diagnosis.
- Understand how drug-resistant mutations (DRMs) are assessed and identified.
- Discuss detection of cytomegalovirus (CMV) drug resistance and mutations in CMV genes.
- Understand how to demonstrate and prove the presence of a virus in a patient.
- Discuss the role of rRNA gene sequencing in pathogen identification.
- Describe genetic α-1 antitrypsin (AAT) deficiency.
- Understand why genetic characterization of AAT deficiency is important in patient management.

1. Next-generation sequencing (NGS) methods comprise several sequencing technologies that have succeeded the traditional dideoxynucleoside chain termination method. Based on the referenced Special article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:623-634.]

a. NGS is also referred to as deep, high-throughput, or massively parallel sequencing.
b. There are several available NGS platforms that differ in their sequencing chemistries, read lengths, and throughput capabilities.
c. With increased accessibility, the NGS platforms have become particularly attractive to clinical microbiology laboratories that already rely on molecular methods for pathogen identification and characterization.
d. Whole genome sequencing (WGS) uses target-specific primers for PCR-mediated amplification.

Rationale: Targeted amplicon sequencing uses target-specific primers for PCR-mediated amplification, so that the genomic regions of interest are enriched and selectively sequenced. This is particularly useful to identify drug-resistant mutants in well-characterized genomic regions. WGS, on the other hand, is useful for *de novo* assembly of whole genomes and relies on nontargeted amplification. WGS is often performed when microorganisms are unknown or to define the genomic content and functional potential of an organism.

2. The emergence of drug resistance is an important factor in the management of several clinically significant viral infections. Based on the referenced Special article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:623-634.]

a. Genotypic drug resistance testing was originally performed using population or bulk sequencing, which involved amplification of specific viral genes followed by Sanger sequencing.
b. The Sanger method has limited sensitivity for minor variants when present at <25% of the viral population, whereas NGS methods can detect drug-resistant mutations (DRMs) present at approximately 5%.
c. HIV-1 is the prototypical virus for NGS-based genotypic resistance testing.
d. Similar to Sanger-based methods, emerging NGS assays have used targeted sequencing of viral genomic regions known to develop resistance mutations.

Rationale: The Sanger method has limited sensitivity for minor variants when present at <15% to 20% of the viral population, whereas NGS methods can detect drug-resistant mutations (DRMs) present at approximately 1%.

3. Epidemiological studies in HIV-1–positive patients have shown that the presence of mutations conferring resistance to highly active antiretroviral therapy can predict treatment outcomes. Based on the referenced Special article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:623-634.]

a. Studies have compared NGS with Sanger sequencing methods for capturing minority-resistant variants, demonstrating that 30% of the DRMs identified by NGS are missed by Sanger sequencing.
b. Genotypic testing for DRMs is currently recommended for therapy-naïve patients when they enter into clinical care and for therapy-experienced patients when they show evidence of virologic failure.
c. A major consideration when assessing minor variants is distinguishing true mutations from artifacts generated during PCR amplification, library preparation, or sequencing.
d. Minor variants include mismatches, insertion/deletions, and PCR-mediated recombination products, known as chimeric sequences.

Rationale: Studies have compared NGS with Sanger sequencing methods for capturing minority-resistant variants, demonstrating that at least half of the DRMs identified by NGS are missed by Sanger sequencing.

4. Genotypic drug resistance testing for cytomegalovirus (CMV) is clinically useful, particularly in transplant recipients. Based on the referenced Special article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:623-634.]

a. Timely detection of CMV drug resistance is critical because DRMs can accumulate with continued exposure to a drug, potentially leading to shortened graft survival and increased morbidity.
b. Rational change of therapy after identification of drug resistance has been shown to lead to more rapid clearance of virus.
c. Rates of CMV drug resistance vary on the basis of patient populations: 5% to 10% in hematopoietic stem cell transplant recipients and 2% to 5% in solid organ transplant recipients.
d. Resistance mutations to current CMV drugs have been characterized in two CMV genes, the DNA polymerase *UL54* and the phosphotransferase *UL97*.

Rationale: Rates of CMV drug resistance vary on the basis of patient populations: 5% to 12.5% in solid organ transplant recipients and 2% to 5% in hematopoietic stem cell transplant recipients.

5. Demonstrating the presence of a virus in a patient with disease does not automatically imply pathogenicity. Based on the referenced Special article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 117:623-634.]

a. Proving that a microorganism is the causative agent of disease has traditionally depended on fulfilling Koch's postulates: a putative etiological agent is found in affected hosts but not healthy controls, it is propagated in culture, and it can reproduce the disease.
b. The ability to culture a virus is a necessary component of proving it is the causative agent of disease.
c. New guidelines have eliminated the requirement for microorganism isolation, but have expanded on the rigor with which the association between microorganism and disease is established.
d. It may be necessary to demonstrate the presence of virus in affected tissues using immunostaining or molecular methods to establish a correlation between viral copy number and disease severity or to show seroconversion from acute to convalescent serum specimens.

Rationale: It is increasingly evident that many viruses cannot be cultured, which has prompted the revision of traditional approaches to prove causality for a microorganism in a disease.

6. rRNA gene sequencing is routinely used for bacterial and fungal identification in clinical microbiology laboratories. Based on the referenced Special article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17:623-634.]

a. For bacteria, sequencing methods use primers targeting conserved 16S rRNA sequences, with variable intervening regions that provide sufficient sequence diversity for taxonomic assignment.
b. The choice of primers is important because certain areas of 16S rRNA genes may allow amplification of a broader spectrum of bacteria than others.
c. Informatics is critical for the interpretation of 16S rRNA sequencing data that are obtained by NGS.
d. The SILVA database contains >400,000 large subunit bacterial rRNA gene sequences.

Rationale: Several extensive databases have been generated for 16S rRNA sequences, including the following: SILVA, containing >3 million small subunit and >250,000 large subunit bacterial rRNA gene sequences, or Greengenes, which can calculate taxonomic relationships on the basis of >400,000 16S rRNA sequences.

7. Genetic α-1 antitrypsin (AAT) deficiency is one of the most common inherited metabolic disorders affecting individuals worldwide. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17: 689-694.]

a. The *SERPINA1 S* and Z alleles are estimated to account for approximately 70% of AAT deficiency alleles.
b. AAT deficiency is underdiagnosed, and appropriate clinical testing is a key component of establishing a definitive diagnosis and identifying carriers.
c. Failure to identify a mutation might contribute to diagnostic and therapeutic delays.
d. Clinical testing is often limited to determining serum AAT levels and targeted mutations analysis for the *S* and *Z* alleles.

Rationale: The *SERPINA1* S and Z alleles are estimated to account for approximately 90% to 95% of AAT deficiency alleles, with other mutations being uncommon.

8. AAT deficiency is characterized by low serum AAT levels and a predilection for early-onset chronic obstructive pulmonary disease and occasionally hepatic disease. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2015, 17: 689-694.]

a. Isoelectric focusing (IEF) electrophoresis is often used to characterize a patient with a low serum AAT level.
b. In current standard clinical testing, genetic AAT deficiency is diagnosed through the detection of low serum levels together with detection of the *S* or *Z* mutations through allele-specific genotyping or proteotyping by liquid chromatography–tandem mass spectrometry.
c. Genetic characterization of an individual with a low serum AAT level is useful to distinguish nongenetic AAT deficiency from genetic AAT deficiency.
d. Genetic characterization of an individual with a low serum AAT level is useful to establish prognosis because some genotypes are associated with only pulmonary symptoms and others with only hepatic symptoms.

Rationale: Genetic characterization of an individual with a low serum AAT level is useful to distinguish nongenetic AAT deficiency from genetic AAT deficiency and to establish prognosis because some genotypes are associated with both pulmonary and hepatic symptoms and others with only pulmonary symptoms.

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