A Review on the molecular analysis of myelodysplastic syndromes, and research articles on next-generation sequencing of autosomal dominant polycystic kidney disease, the detection of Clostridium difficile, and the detection of Salmonella enterica species were selected for the March 2014 JMD CME Program in Molecular Diagnostics. The authors of the referenced articles, the planning committee members, and staff have no relevant financial relationships with commercial interests to disclose.


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

• Discuss myelodysplastic syndromes (MDS) and the affected population.
• Understand the molecular basis of MDS.
• Describe the use of conventional karyotyping and single nucleotide polymorphism arrays in MDS diagnosis.
• Understand the role of single gene molecular alterations in the development of MDS.
• Define MDS DNA methylation status.
• Describe autosomal dominant polycystic kidney disease (ADPKD).
• Understand the genetic basis of ADPKD.
• Describe Clostridium difficile and the current methods for its diagnosis.
• Understand Salmonella enterica species infections and how they are diagnosed.

1. The myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders of ineffective hematopoiesis. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:145-158.]

   a. MDS characteristically demonstrate peripheral blood cytopenia, bone marrow hypercellularity, and morphologically defined dysplasia of one or more hematopoietic lineages.
   b. MDS typically affect adults with a median age of 55 years at diagnosis.
   c. MDS are recognized as causes of bone marrow failure in the pediatric setting.
   d. MDS was once thought of as almost invariably leading to the development of an acute leukemia; however, our understanding of these diseases as a distinct group of disorders has evolved with the recognition that a majority of cases never progress to acute myeloid leukemia (AML).
2. The molecular basis for MDS is only beginning to be elucidated, and unifying themes remain elusive, although epigenetic and spliceosome pathways are emerging as frequent targets. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:145-158.]

   a. Flow cytometry no longer plays a role in the diagnosis of MDS.
   b. Genetic alterations in hematopoietic precursors, likely including stem cells, undoubtedly underlie the distinct natural disease course of MDS subtypes.
   c. A diverse group of tests have been developed to test for the molecular changes in MDS, for example, mutations, miRNA, and altered methylation states.
   d. Understanding MDS development at a mechanistic level will aid in diagnosis, determination of prognosis, and the generation of novel, directed therapies.

3. Conventional karyotyping, performed on metaphase cells with a Giemsa (G) stain, enables a coarse but very useful genome-wide survey. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:145-158.]

   a. The G-banding of 20 metaphase cells is evaluated, revealing most large translocations, gains, and deletions.
   b. The karyotype is central to the diagnosis and accurate classification of MDS and essential for prognostication.
   c. Karyotypic abnormalities, detected by metaphase chromosomal analysis in approximately 65% of all cases of de novo MDS, are less frequent in patients with secondary MDS (~40%).
   d. The partial or complete loss of chromosomes followed by partial or complete gains are most characteristic of MDS.

4. Single nucleotide polymorphism (SNP) arrays, with the ability to sensitively detect loss of heterozygosity in tumors, appear to be a useful addition to metaphase cytogenetics by capturing additional cryptic gains or losses. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:145-158.]

   a. Use of SNP arrays results in the discovery of more chromosomal abnormalities than by metaphase cytogenetics alone. In patients with normal cytogenetic profiles, the cryptic changes discovered by SNP arrays are often adverse prognostic indicators.
   b. In diagnostic MDS specimens, SNP arrays are already starting to become a complementary method for conventional cytogenetics with their own prognostic import.
   c. SNP array has been used to distinguish hypocellular MDS from aplastic anemia.
   d. SNP analysis is used to detect balanced chromosomal translocations.

5. Single gene molecular alterations in MDS are currently being elucidated at a rapid pace. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:145-158.]

   a. About 80% of MDS patients have detectable mutations, but no specific mutation is present in more than about 40% of patients with MDS.
   b. The vast majority of MDS mutations are low incidence.
   c. Cases of secondary MDS are more likely to have mutations than cases of de novo MDS.
   d. A wide variety of mutations have been identified, including TET2, RUNX1, TP53, NRAS, ASXL1, and less commonly CBL and EZH2.

6. The two most prominent mechanisms in MDS, DNA methylation and histone acetylation and methylation, also play roles in physiologic hematopoiesis. Based on the referenced Review, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:145-158.]

   a. Seventy percent of high-risk MDS patients had hypermethylation of ALOX12, GSTM1, HIC1, FZD9, and HS3ST2.
   b. MDS is associated on a genomic level with a global increase in methylation.
   c. A number of genes that encode proteins that modulate the epigenetic status, including IDH1/IDH2, TET2, and DNMT3A, are mutated in myeloid malignancies.
   d. Although there are strategies for assaying methylation status in other conditions, there is no current standardized clinical assay in MDS.

7. Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in PKD1 and PKD2. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:216-228.]

   a. ADPKD affects 1 in 50 to 1 in 500 live births worldwide.
   b. ADPKD is the most common inherited kidney disease.
   c. ADPKD accounts for approximately 5% of the end-stage renal disease population.
   d. ADPKD is initiated by gene mutations in renal tubular epithelial cells.
8. The genetic analysis of ADPKD is complicated by six \textit{PKD1} pseudogenes, large gene sizes, and allelic heterogeneity. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:216-228.]

   a. \textit{PKD1} and \textit{PKD2} account for 75% to 85% and 15% to 25% of cases of ADPKD, respectively.
   b. \textit{PKD1} spans 46 exons and encodes polycystin-1 with 4,303 amino acids.
   c. Chromosome 16 includes six homologous genes that share 97.7% sequence identity with the \textit{PKD1} gene exons 1 to 33.
   d. \textit{PKD2} spans 9 exons, encoding polycystin-2, which consists of 1,005 amino acids.

9. \textit{Clostridium difficile} is an anaerobic, spore-forming, Gram-positive bacterium that colonizes the human colon. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:244-252.]

   a. \textit{C. difficile} typically presents as an opportunistic infection after other colonic flora have been eradicated by commonly used antibiotics.
   b. \textit{C. difficile} infection can cause severe diarrhea, pseudomembranous colitis, and toxic megacolon, and may require urgent colectomy or result in death.
   c. \textit{C. difficile} infection is rapidly increasing in incidence, severity, and mortality, particularly in the United States, Canada, and Europe.
   d. In the United States, \textit{C. difficile} infection was responsible for $2.5\text{ billion in excess hospital costs in 2008 and an estimated 10,000 deaths from 2006 to 2007.}$

10. The changing epidemiology of \textit{C. difficile} has been marked by hospital outbreaks due to a hypervirulent strain, NAP1/027/B1. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:244-252.]

   a. Current methods of diagnosing \textit{C. difficile} include stool culture, toxin testing, enzyme immunoassays, and PCR.
   b. Stool culture and cytotoxicity tests provide high sensitivity and specificity.
   c. Stool culture and cytotoxicity tests require at least 7 days to complete.
   d. Several multiplex PCR assays have been reported to identify various genes associated with \textit{C. difficile}.

11. \textit{Salmonella enterica} species infections are a significant public health problem. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:261-266.]

   a. Each year in the United States, approximately 1 million domestically acquired foodborne illnesses and >350 deaths occur because of nontyphoidal \textit{S. enterica} species infections.
   b. Approximately 52.9 million illnesses and 250,000 deaths occur annually worldwide from nontyphoidal \textit{S. enterica} species infections.
   c. Typhoidal \textit{S. enterica} species infections cause approximately 21.7 million cases of typhoid fever and >200,000 deaths annually worldwide.
   d. Typhoidal and nontyphoidal \textit{S. enterica} species cause high worldwide morbidity rates and high mortality rates in the developing world.

12. The global burden of \textit{S. enterica} infections is poorly characterized due, in part, to insufficient diagnostic and surveillance methods and emergence of antimicrobial-resistant \textit{S. enterica} species. Based on the referenced article, select the ONE statement that is NOT true: [See J Mol Diagn 2014, 16:261-266.]

   a. The Centers for Disease Control and Prevention and the World Health Organization have established laboratory-based surveillance programs and guidelines for the detection, identification, treatment, and prevention of \textit{S. enterica} species infections.
   b. Not all \textit{S. enterica} species infections are properly diagnosed, leading to delays in adequate treatment and accurate surveillance data.
   c. Feces are the only source material from which the diagnosis of an \textit{S. enterica} species infection can be made in a clinical laboratory.
   d. 16S PCR coupled with high-resolution melt analysis could be a useful molecular diagnostic method to enhance the current diagnostic, treatment, and surveillance methods of \textit{S. enterica} bloodstream infections.