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](http://dx.doi.org/10.1016/j.jmoldx.2015.01.003)


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.]

   - 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.
   - Genetic and genomic testing is clinically available for >8000 genetic conditions.
   - Genetic and genomic testing, although fairly low volume relative to other laboratory tests, contributes substantial cost to laboratory medicine.
   - The substantial cost to laboratory medicine is in part because of the increasing availability and complexity of molecular test options.

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 study of United Healthcare members found that spending on molecular genetic tests increased 34% per year between 2008 and 2010.
   - 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.
   - 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.
   - 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.
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.]

- 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).
- 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.
- At the present time, nearly 100 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.]

- 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.
- 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.
- The clinical syndrome often dictates the sites of specimen collection.
- Throat swab 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.]

- 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.
- Successful visualization of virus by EM requires a virus concentration of $10^2$ to $10^3$ particles/mL of specimen.
- 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.
- 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.

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.]

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

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.]

- Oligonucleotide adaptor ligation and adaptor-specific PCR amplifies both cDNA populations (tester and wild-type).
- Hybridization of tester with adaptor-ligated driver DNA follows the ligation and PCR steps.
- 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.
- The discovery of polyomavirus was made using the RDA method.

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.]

- RCA has been used for the discovery of circular DNA viruses such as herpesviruses.
- RCA protocols for the detection of novel viruses use multiple random hexamer primers that bind to different points of the circular genome.
- 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.
- 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.