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Three research articles on the use of the Idylla system for rapid molecular testing of variant allele frequency, the use of
PipeIT for variant calling for Ion Torrent sequencing, and the optimization of population frequency cutoffs for filtering
common germline polymorphisms from tumor-only next-generation sequencing data were selected for the September 2019
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: Huang H, Springborn S, Haug K, Bartow K, Samra H, Menon S, Mackinnon AC; Evaluation,
Validation, and Implementation of the Idylla System as Rapid Molecular Testing for Precision Medicine. J Mol Diagn 2019,
21: 862-872; https://doi.org/10.1016/j.jmoldx.2019.05.007

Questions #5-8 are based on: Garofoli A, Paradiso V, Montazeri H, Jermann PM, Roma G, Tornillo L, Terracciano LM,
Piscuoglio S, Ng CKY; PipeIT: A Singularity Container for Molecular Diagnostic Somatic Variant Calling on the Ion Torrent

Questions #9-12 are based on: McNulty SN, Parikh BA, Duncavage EJ, Heusel JW, Pfeifer JD; Optimization of Population
Frequency Cutoffs for Filtering Common Germline Polymorphisms from Tumor-Only Next-Generation Sequencing Data. J
Mol Diagn 2019, 21: 903-912; https://doi.org/10.1016/j.jmoldx.2019.05.005

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

• Discuss the validation, use, implementation as well as the limitations of the Idylla testing system.
• Discuss the use of PipeIT for variant calling for Ion Torrent sequencing.
• Understand the advantages of using container technologies for inter-laboratory validation using next-generation
  sequencing bioinformatics assays.
• Understand the significance of distinguishing germline from somatic variants in cancer testing.
• Understand the importance of population frequency cutoffs in determining the status of germline versus somatic
  variants.

1. The Idylla Mutation Test System is a fully automated, PCR-based mutation testing system. The advantages of
   this system can greatly impact the delivery of precision medicine. Based on the referenced article, select the
   a. The Idylla test is fairly comparable to the conventional standard of care (SOC) methods used to identify clinically
      actionable genomic biomarkers.
   b. Both SOC testing and the Idylla system have difficulties processing challenging archived specimens.
   c. Unlike the long turnaround time (TAT) for SOC testing, the Idylla system only requires a few hours from tissue
      input to result.
   d. Though accessible and rapid, Idylla is far from being a robust and reliable testing option.

2. The limit of detection (LOD) is defined as the lowest allelic frequency at which the mutant alleles can
   consistently be detected in ≥95% of the test cases. To estimate the LOD for the mutations detected by the
   Idylla tests, a set of commercially available formalin-fixed, paraffin-embedded (FFPE) reference samples with
   1%, 5%, and 50% known mutant variant allele frequency (VAF) were tested to verify the LOD for each mutation.
   Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 862-872]
   a. The Idylla test can detect an LOD of 1% with 100% CI for BRAF V600E and V600K, and EGFR L861Q, E746 to
      A750, L858R, and G719S.
   b. The Idylla test can detect an LOD of 1% with 100% CI for KRAS G12A, G12C, G12D, G12R, G12S, G12V,
      and G13D.
   c. The Idylla test can detect an LOD of 5% with 100% CI for KRAS G12A.
   d. The Idylla test can detect an LOD of 1% with 100% CI for EGFR T790M.
3. The TAT is defined as the time from when the sample was received in the laboratory to when the results became available to the clinician. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 862-872]
   a. The TAT for molecular testing of pathology specimens is fairly consistent for various testing methods.
   b. The TAT includes sample preparation, DNA extraction, sample loading, test performance, data analysis, and report.
   c. The average SOC TAT ranges from 7 to 15 days, depending on the site (in-house or send-out) and method.
   d. The typical TAT for the Idylla mutation test is within 3 to 5 days.

4. The Idylla system has some limitations. Based on the referenced article, select the ONE best TRUE statement:
   [J Mol Diagn 2019, 21: 862-872]
   a. The Idylla system can discern true-negative results from false-negative results, which may be caused by secondary mutations that can affect the PlexZyme/PlexPrime technology used by the cartridge.
   b. Idylla false negative results are attributed to VAF below the threshold detectable by Idylla, rare mutations not included in the Idylla test references range, and technical problems related to malfunctioning cartridges.
   c. The Idylla system may still detect some low amounts of EGFR T790M mutations.
   d. Despite limitations, the Idylla system is highly scalable.

5. Ion Torrent sequencers are most frequently used for surveying cancer mutation hotspots and/or a limited number of cancer genes in molecular diagnostics laboratories. However, there is a lack of consensus on how to perform somatic mutation analysis for Ion Torrent data. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 884-894]
   a. A typical approach to perform somatic mutation calling on the Ion Torrent platform is through the proprietary browser-based Ion Reporter (IR) interface.
   b. The specificity of the tools not designed to consider the Ion Torrent–specific flow space is comparable to the specificity of Torrent Variant Caller (TVC), the underlying variant calling engine of the IR.
   c. IR suffers from an approximately 30% false-positive (FP) rate.
   d. IR analysis support for both the commercially released Ion Torrent assays and targeted sequencing panels is pretty extensive.

6. To ensure reproducibility and to ease software deployment using next-generation sequencing (NGS) bioinformatics pipelines, container technologies are being adopted by the bioinformatics community as prebuilt packages in which the necessary software is already installed, tested, and ready to be executed. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 884-894]
   a. A container technology pipeline has not proved effective in transferring NGS analysis pipelines between laboratories.
   b. Docker containers are found for many commonly used bioinformatics tools but it cannot be considered the gold standard of container technologies.
   c. Docker images usually require root privileges to be executed, making them impractical for regular users in shared high-performance computing clusters.
   d. Singularity is the gold standard of container technologies.

7. Modern clinical molecular diagnostics are becoming increasingly reliant on the identification of somatic genetic alterations using NGS. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 884-894]
   a. The Ion Torrent platform is one of the main sequencing platforms used in the clinical setting but it suffers from high costs and slow TAT.
   b. PipeIT is a Singularity container for diagnostic somatic variant calling on the Ion Torrent platform that is applicable only for Oncomine panels.
   c. The PipeIT workflow can only be run from start to finish.
   d. Singularity container technology ensures reproducibility of results between laboratories.

8. The performance of PipeIT was demonstrated using two data sets. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 884-894]
   a. In the first set of 15 FFPE colon adenomas sequenced using the Oncomine Comprehensive Panel, PipeIT failed to identify the bona fide pathogenic mutations identified by IR-Oncomine.
   b. PipeIT correctly identified all germline variants called by IR-Oncomine.
   c. PipeIT identified all IR–tumor-normal (IR-TN)–specific variants enriched for low VAF and/or low depth.
   d. In 10 fresh-frozen hepatocellular carcinomas (HCCs) sequenced using a custom AmpliSeq panel, PipeIT has excellent positive predictive value (PPV) compared with IR solutions.

9. Cancer cells contain both inherited, germline polymorphisms and tumor-specific somatic mutations that are either the cause or consequence of unchecked cell division. Based on the referenced article, select the ONE best TRUE statement: [J Mol Diagn 2019, 21: 903-912]
a. Distinguishing germline from somatic variants is an important aspect of cancer testing.
b. Paired tumor-normal sequencing may sometime allow determining the germline or somatic origin of a variant.
c. Variants present in both specimens are somatic and the variants unique to the tumor are germline.
d. Paired tumor-normal sequencing is common in clinical practice due to low costs and ease of reimbursement.

10. Clinical NGS assays are often run on tumor specimens without a matched normal specimen, which complicates the differentiation of germline from somatic variants. Based on the referenced article, select the **ONE best TRUE statement**: [J Mol Diagn 2019, 21: 903-912]
   a. In tumor-only testing, population data are often used to infer germline status; there is some established consensus on the exact population frequency (PF) cutoff above which a variant should be considered likely germline.
   b. The 1% to 2% PF cutoffs widely used in bioinformatic pipelines resulted in high sensitivity for classification of somatic variants, but unnecessarily reduced sensitivity for germline variants.
   c. Using optimized PF cutoffs, the source of variants in The Cancer Genome Atlas (TCGA) data could be predicted with 100% accuracy.
   d. TCGA cancer data sets indicated that the optimal cutoff is influenced by only cancer type and is independent of the assay region of interest (ROI).

11. The Association for Molecular Pathology/American Society of Clinical Oncology/College of American Pathologists working group stressed the importance of distinguishing somatic from germline variants, and recommended potential strategies to infer a variant’s origin. Based on previous findings and the referenced article, select the **ONE best TRUE statement**: [J Mol Diagn 2019, 21: 903-912]
   a. Germline variants, present in every cell in the body, should have variant allele fractions of approximately 100% for heterozygous variants.
   b. Germline variants, present in every cell in the body, should have variant allele fractions of approximately 50% for homozygous variants.
   c. The variant allele fractions of germline variants, present in every cell in the body, remains constant.
   d. Technical and biological factors can influence reporting of the variant allele fractions of germline variants.

12. With careful optimization of population frequency cutoffs, germline versus somatic status of variants can be estimated with high sensitivity and specificity. Based on the referenced article, select the **ONE best TRUE statement**: [J Mol Diagn 2019, 21: 903-912]
   a. The PF threshold is independent of the genomic ROI.
   b. The PF threshold is independent of the technical methods.
   c. The careful optimization of PF cutoff can improve the sensitivity and specificity of variant classification in tumor-only NGS data.
   d. The sensitivity and specificity of variant classification is independent of the customization of PF cutoff for individual NGS tests.