

Precision in Action: Reliable Performance and Validation of Hereditary Cancer Panel Testing for Superior Clinical Confidence

Introduction

In the rapidly evolving field of diagnostic testing, the accuracy, reliability, and reproducibility of assays are paramount to ensuring patient safety and clinical decision-making. Assay validation is the cornerstone of establishing these critical characteristics, serving to systematically evaluate the performance of a diagnostic test. This process is essential not only for meeting regulatory requirements, but also for providing healthcare providers and patients with confidence in test results.

In a commercial diagnostic laboratory, where assays are routinely used to assess patient health, the stakes are particularly high. The increasing applications of personalized medicine, and the rapid advances in molecular diagnostics technologies and point-of-care testing, have underscored the need for rigorous assay validation (Figure 1). A validated assay ensures that it consistently delivers accurate and reproducible results across a range of clinical conditions and patient populations.

Furthermore, assay validation serves as a safeguard against false positives, false negatives, and other diagnostic errors that could lead to incorrect treatment decisions.

CancerNext® is a guideline-based multigene panel test that includes genes that are known to have clinical management recommendations for detection, prevention, and/or risk reduction.

This whitepaper describes the validation of a multigene panel test, CancerNext, designed to identify individuals with germline cancer predisposition. Key validation principles and the potential risks of inadequate validation will be highlighted. By examining best practices in assay development and implementation, we aim to underscore the role of assay validation in maintaining high standards of diagnostic accuracy and reliability.

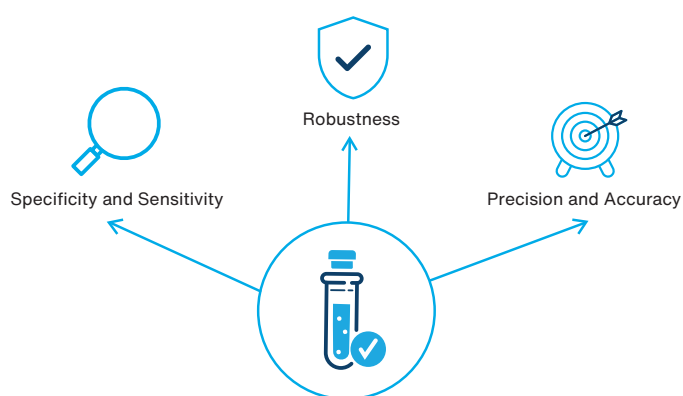
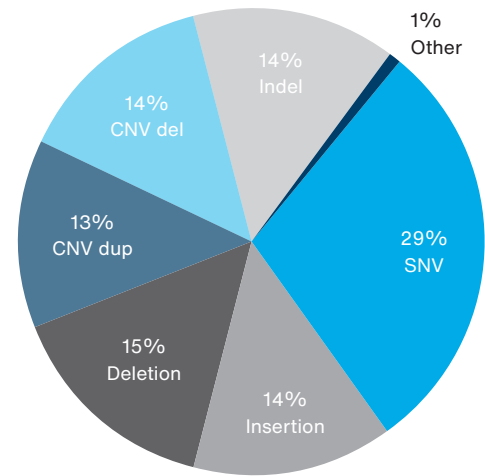


Figure 1. Fundamentals of Assay Validation

Validation Across Diverse Specimen and Variant Types

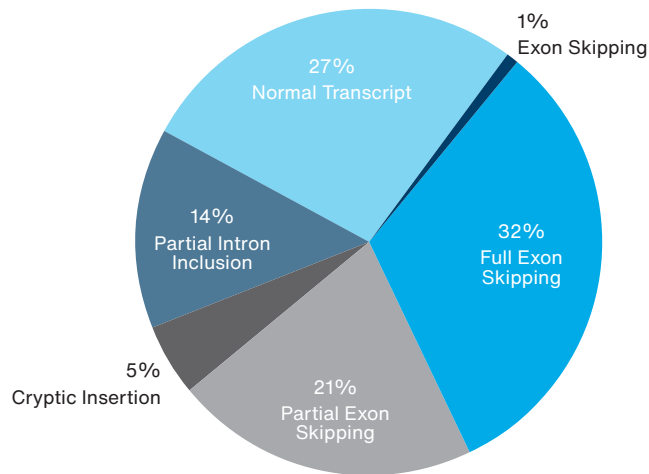
The validation of genetic tests across multiple specimen and variant types is critical to ensure the reliability and applicability of the test in diverse clinical scenarios. Different specimen types, such as blood, saliva, or buccal, can vary significantly in DNA quality, quantity, and composition. For a genetic test to be universally applicable, it must perform consistently across these specimens, accounting for any variations in DNA extraction methods, potential inhibitors, or degradation of genetic material.

In addition, genetic variants themselves can exhibit a wide range of characteristics, including single nucleotide variants (SNVs), insertions/deletions (indels), and copy number variations (CNVs) on the DNA level (Figure 2). With regards to RNA, abnormal transcripts can arise from a variety of splicing events, including full and partial exon skipping, cryptic insertion, and intron inclusion (Figure 3). These variations may have different effects on the sensitivity and specificity of genetic testing platforms. Validating a genetic test across these variant types ensures that it can detect a broad spectrum of genetic alterations without bias, providing comprehensive and accurate results.



100% Concordant with Existing Assay
n=818 samples

Figure 2. Types of DNA Variants Evaluated



99.6% Concordant with Existing Assay
n=555 samples

Figure 3. Types of RNA Splicing Events Evaluated

Evaluation of Concordance

Intra-Assay Repeatability

Intra-assay repeatability refers to the consistency of results obtained when the same sample is tested multiple times within a single assay run, typically under the same conditions (e.g., same equipment, same technician, same reagents). It assesses the ability of the assay to produce identical or very similar results under the same set of operational conditions (Figure 4).

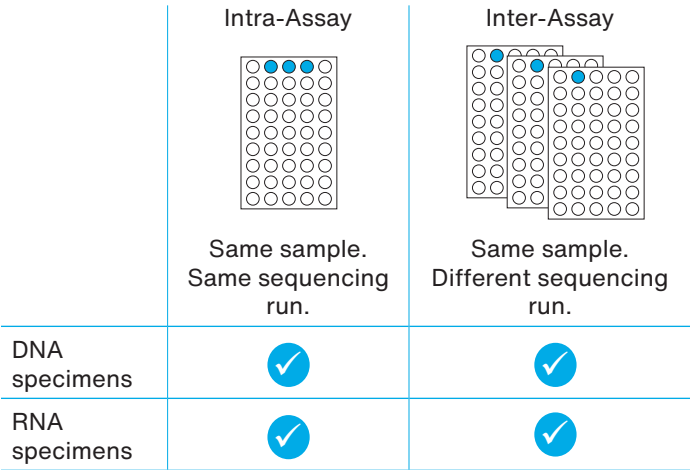


Figure 4. Intra-Assay Repeatability and Inter-Assay Reproducibility

Intra-assay repeatability is evaluated to determine the precision of the assay in a single experiment or test. High intra-assay repeatability suggests that the assay can reliably produce consistent results from the same sample when performed in the same session.

Inter-Assay Reproducibility

On the other hand, inter-assay reproducibility, evaluates the consistency of results when the same sample is tested across multiple assay runs, often performed on different days, by different technicians, using the different instruments associated with the test, or with different reagent lots. It measures the variability of results across different conditions and over time, reflecting the assay’s robustness and its ability to provide consistent performance across different operational variables (Figure 4). This parameter is crucial for assessing the assay’s reliability and stability. High inter-assay reproducibility indicates that the assay is robust and can withstand variation in operating conditions over time.

Meeting Consistent Thresholds

Maintaining high-quality data throughout the entire genetic testing workflow is crucial for ensuring the most accurate results. This process is typically divided into three distinct stages: pre-analytical, analytical, and post-analytical. Following analysis, the clinical significance of any variants detected is then assigned. Each stage requires the development of specific thresholds to determine the viability of the sample and result before subsequent steps in the workflow can occur (Figure 5).

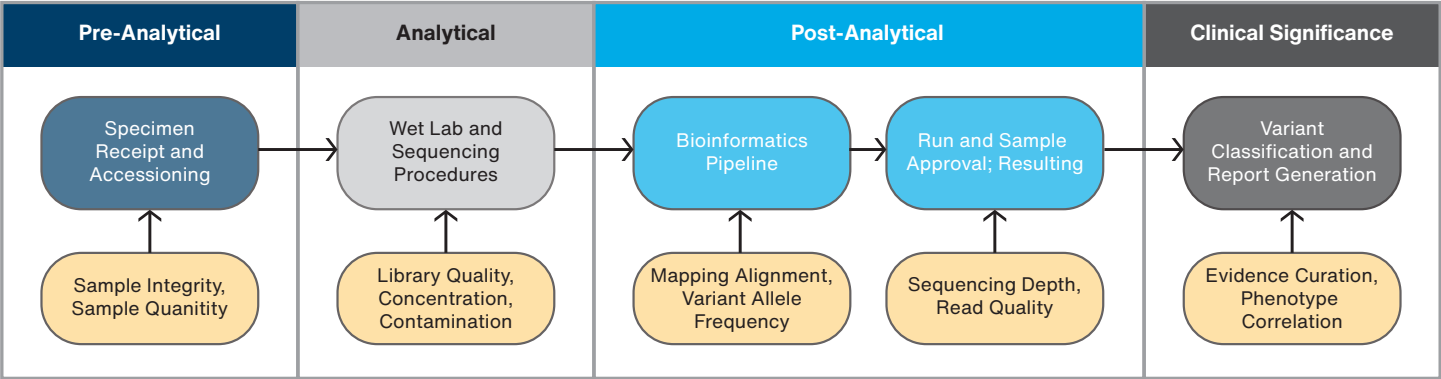


Figure 5. Examples of Quality and Reporting Thresholds in an NGS Workflow

When a sample is prepared for processing upon its initial receipt, the DNA concentration and quality are measured before it can move on to sequencing. During the analytical phase, concentration and contamination limits are measured after the library is prepared. In the post-analytical stage, the vast amount of genomic data generated during sequencing is organized by a bioinformatics pipeline, aligned to a reference genome, and filtered based on variant allele frequency. Finally, the sample and results must be approved before reporting. Specific sequencing criteria for coverage depth and read quality must be met. Regions not satisfying these criteria are verified by Sanger sequencing, or in some cases, samples may be entirely re-processed.

Failure to meet quality metrics or reporting thresholds at any one stage will flag the case for follow-up. Establishing these thresholds allows laboratories to monitor and mitigate errors that could affect downstream analysis and interpretation.

Conclusions

As demonstrated in the validation of the CancerNext multigene panel test, a comprehensive approach that addresses key aspects such as specimen diversity, variant types, repeatability, and reproducibility is essential for ensuring consistent performance across various clinical scenarios. By rigorously assessing the test's ability to meet established thresholds across the entire testing workflow, laboratories can safeguard against diagnostic errors and enhance patient care. These validation practices not only meet regulatory requirements but also foster trust and confidence in genetic testing, ultimately supporting better clinical decision-making and improved patient outcomes.

References

1. Clinical Laboratory Improvement Amendments (CLIA), 42 CFR Part 493.
2. Bean LJH, et al. ACMG Laboratory Quality Assurance Committee. Diagnostic gene sequencing panels: from design to report-a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2020 Mar;22(3):453-461.
3. Mu W, et al. Sanger Confirmation Is Required to Achieve Optimal Sensitivity and Specificity in Next-Generation Sequencing Panel Testing. *J Mol Diagn*. 2016 Nov;18(6):923-932.