

Automate the QC Data Analysis of Your Clinical NGS Test

Save Time and Money Collecting and Organizing QC Data for Audits and Troubleshooting

Today's clinical laboratory is a complex operation. With the advent of high-throughput next-generation sequencing (NGS), a cost-effective and rapid method of genetic testing for multiple genes simultaneously for cancer mutations or for genetic disorders is now routine.

Francine de Abreu, Assistant Director, Clinical Genomics and Advanced Technology (CGAT) of Dartmouth-Hitchcock Medical Center shared her insight in a corporate workshop at the Association for Molecular Pathology's annual conference. In the CGAT lab, they employ NGS in routine clinical use and have transitioned over from a bottleneck-generating, manual NGS QC to a streamlined, automated NGS QC. She discussed several ways in which using automated QC tracking software is critical for high-throughput validation and routine clinical use.

NGS Testing at Dartmouth-Hitchcock Medical Center

In the CGAT Laboratory at Dartmouth, they have implemented NGS tests for oncology and inherited disease for several years. "There are many panels you can use to detect genetic diseases, but more importantly, you can develop your own customized panel based on the disease you are looking for," says de Abreu.

The CGAT lab has a joint clinical and research focus and has CAP and CLIA certifications. The College of American Pathologists (CAP's) Laboratory Accreditation Program accredits all laboratory test disciplines with their comprehensive, scientifically endorsed laboratory standards, which helps laboratories maintain accuracy of test results and ensure accurate patient diagnosis. In the US, the Clinical Laboratory Improvement Amendments (CLIA) program regulates laboratory testing and requires clinical laboratories to be certified by their state as well as the Center for Medicare and Medicaid Services (CMS) before they can accept human samples for diagnostic testing.

The lab offers specific oncology panels on the Ion Torrent® PGM® platform, such as the Ion AmpliSeq® Cancer Hotspot Panel v2 (CHPv2), designed to amplify genomic "hot spot" regions that are frequently mutated in human cancer genes at the DNA level, and the RNA-based Archer® FusionPlex® Solid Tumor Panel, designed to characterize fusions and mutations of over 50 genes linked to various solid tumors, including carcinomas, sarcomas, and lymphomas. They also run inherited disease panels for conditions such as cardiomyopathy and inborn metabolism disorders.

Developing QC Metrics During Validation

The choice of genes to include in a given panel, as well as its feasibility and implementation, are carefully considered factors. Once these decisions have been made, they prepare for validation, which involves “some things for which we have no guidelines but are very important,” says de Abreu. “You decide during your validation on all of the QC metrics you would like to use during your workflow. Once these are established, you will track these during your routine clinical run,” she says.

Metrics for sensitivity, specificity, reproducibility, and accuracy are the most important metrics for an NGS validation; however, there are many additional parameters and limits that need to be measured for each phase of sequencing analysis as shown in Table 1.

NGS Sequencing Analysis

Extraction, Quality, and Quantification
- Evaluate number of unstained slides
- Evaluate percent of tumor content
- Establish method and limits for nucleic acid quantification
- Establish method and limits for nucleic acid quality

Library Preparation
- Establish limit of detection (LOD) of nucleic acid input
- Establish LOD of allele frequency
- Establish method and limits for library quantification

Sequencing
- Establish run metrics for the NGS instrument: Chip loading
- Establish run metrics for the NGS instrument: Usable sequences
- Establish run metrics for the NGS instrument: Polyclonality
- Establish run metrics for the NGS instrument: Low quality reads

Data Analysis and Curation
- Establish sample metrics: On-target reads
- Establish sample metrics: Uniformity
- Establish sample metrics: % amplicon drop-out
- Establish variant metrics: Presence/absence
- Establish variant metrics: Allele frequency

Table 1. Determination during validation

Poor sample quality was highlighted by Dr. de Abreu, as it affects the specificity and sensitivity of detecting a mutation. “If you have low uniformity and high amplicon dropouts, you might get false negatives. This means you have (poor) sample quality,” says de Abreu.

Post-Validation Daily Run QC Challenges

“The problem is not just with tech time, but with turnaround time. What becomes a problem is... when you are increasing turnaround time and the clinicians are calling and the patients are waiting to get their results so they can be treated,” says de Abreu. She recounted one time, while using manual QC, they had a reagent which was failing repeatedly. Their normal turnaround time was seven days, and in that case they took 14 days to identify the problem, re-run the test, and then report the result, which was a problem “because the patient was waiting.”

By manually tracking QC parameters using a spreadsheet, they had to take several days to collect assorted QC variables, including reagent lot numbers, to determine the root cause of the assay failure. Dr. de Abreu put it this way: “Instead of having 3-4 steps for sequencing where they are not talking to each other, we wanted instead a unified QC track. What we were looking for was a way to (monitor) problems throughout the workflow.”

Solution: SeraCare™ iQ NGS QC Management Software

QC management software is a powerful improvement over labor-intensive manual QC for NGS applications, with a significantly more efficient and higher-quality method of tracking and trending QC data. SeraCare’s [iQ NGS QC Management software](#) allows the examination of several user-defined parameters at a glance, with the potential to prevent blind spots and save precious time to determine the source of failure with a given run. The software also makes being ready for audits as easy as clicking a button to generate a lab QC report that shows when metrics were signed off and by whom.

“The only way to save time and prevent the frustration of manual methods is to stop using manual tracking and start using automated tracking. That’s why we started using SeraCare’s iQ NGS QC Management software,” says de Abreu. Their laboratory’s manual method involved tracking individual values across separate spreadsheet and hard-copy files, with no way to quickly associate problems from a particular run result with a given lot number, instrument, or operator.

The ability to track real-time QC metrics is “one of the best features because you can see what’s going on with your run,” says de Abreu. In addition, the ability to customize QC tracking pipelines for the various types of software is of value. “In our lab, we have a total of six QC (pipelines),” she added.

The software provides information on run statistics such as pass/fail for each parameter, and statistics on each variant’s allele frequency.

Another benefit is that you can view what’s happening in each run with different lot numbers, helping you to visualize trends, changes, and to flag any problems.

“One of the best features of the software besides tracking for the things you want is the ability to track things that you didn’t even know existed until you started tracking them,” says de Abreu.

With iQ NGS QC Management, it is now more feasible for de Abreu and her team to track over 14 different data points per sample and run to help ensure the performance of their assay and identify drift.

After your sequencing run, iQ NGS QC Management allows you to easily review and approve run data and print an inspection-ready report. With informative graphics, variables can be monitored individually or together by simply clicking on variables of interest, which can help you quickly determine which of your QC metrics passed or failed. The run data can then be exported as a spreadsheet file or saved as a PDF report.

In addition, multiple individuals can be assigned various levels of authentication (i.e. users or administrators). See Table 2 for a summary of these features.

Feature	Benefit
Customized QC tracking pipelines	Individual assay characteristics have their own set of QC parameters
QC metrics tracked in real-time	Fast access to important data parameters, reduce assay downtime
Visualization of performance data across multiple runs	Identify trends over time
Automated extraction of variant frequency data from VCF files	Save time in calculating QC allele frequencies
Visualization of multiple user-defined parameters	Quickly troubleshoot root cause or identify correlations

Table 2. Features and Benefits of the SeraCare iQ NGS QC Management software

Summary

With iQ NGS QC, “you can go beyond what you originally intended, and gain insight into variables you didn’t even know you wanted to look at,” says de Abreu. By providing insight into previously unexamined variables, the end result is faster and higher quality results for routine clinical use and research applications.

[Sign up and start saving time tracking QC metrics now with a free evaluation of iQ NGS QC Management](#)

Free 30-day trial!