Finally, Reliable Quality Control for NGS Labs

How SeraCare's Reference Material Ensures Diagnostic Results You Can Count On

If you're a lab director or pathologist for a clinical genomics laboratory, we don't have to tell you how much is riding on your test results. If you're like most people in your position, your obsession with your lab's results consumes you. Two overwhelming urges, in particular, drive you:

1. That every single result your lab reports to clinicians and patients is consistently accurate, day in and day out.
2. That your quality control (QC) program catches mistakes, reagent failures, instrument performance issues, and assay performance drift before they get out of hand and cause expensive downtime or ultimately cause you to report an inaccurate result. For you, anything short is the stuff of nightmares. And considering your lab's weighty responsibility in patient care — and by extension *your responsibility* as a lab director — why wouldn't it keep you tossing and turning at night?

Accurate results matter because:	**Catching problems proactively matters because**
Directing the wrong treatment can put patients' lives and wellbeing at risk and drive up unnecessary healthcare costs.	Your lab can't afford to shut down a test to troubleshoot performance problems. It costs time, personnel, and money.
Missing a diagnosis can delay appropriate treatment and allow diseases to progress.	With your highly-complex next-generation sequencing (NGS) assays, there are many different points in the process where something could go wrong. These problems are hard to find and can be magnified over the hundreds of samples you run every day.
Your lab must adhere to quality and regulatory standards (CAP/CLIA in the U.S., ISO 15189 in Europe). Running afoul of your lab's accreditation and licensing status could cause a shut down, impacting all the other tests you provide.	Day-to-day variants can impact an assay's ability to resolve a positive when it is near the assay's limit of detection (LOD). You need to be sure your assays are sensitive enough to catch these cases.
Your lab's hard-won reputation is one of your most precious assets. Any damage to it could harm your operations and the livelihood of those who work for it.	

Quality Control: Helping Lab Directors Sleep at Night

With worries about accuracy and consistency threatening your peace of mind, your laboratory's quality control program provides some measure of relief. It's your backup against assay performance issues — or worse, reporting the wrong result.

Most clinical genomics labs have a quality control program that includes developing and tracking key metrics in the assay workflow. Some include the use of a run control to monitor assay performance. As we advocated in our "2 Tools For Overcoming Your Clinical Lab's Toughest Quality Control Challenges," a robust and effective quality control program — that is, one you can rely on — includes both strategies.

In the next section of this guide, we'll focus on monitoring assay performance by running controls. We'll compare the three most common approaches to this critical element of quality control. And finally, we'll explain how SeraCare's Seraseq™ tools for clinical genomics QC, can help put your worries about inaccurate results to rest with a range of high-quality, patient-like reference materials designed to detect just about any variant type or variant frequency your assay is designed for.

3 QC Solutions for Clinical Genomics Labs: The Pros and Cons

When it comes to the use of quality control reference material, lab directors usually choose one of three paths:

1. **Not running a control**, trusting in the thoroughness of their validations, metric tracking, and strict adherence to standard operating procedures.
2. **Developing their own reference materials in-house**, also known as the "homebrew" solution.
3. **Obtaining reference material from a commercial vendor**.

Which path will work best for your lab? Let's compare each one.

1. Not Running Controls

Both the College of American Pathologists (CAP) and the Association for Molecular Pathology (AMP) in their recent guidance for validation advise running positive and negative controls. Yet, often to save money or space, some labs choose to overlook these recommendations.

> ***"Control samples can be used to readily detect sources of error and avoid potential harm to the patient. These should be used to monitor steps of the assay when validation data are insufficient to ensure that potential errors are exceedingly unlikely to occur."***
>
> ***-Guidelines for Validation of NGS-based Oncology Panels***

When labs don't run controls, they rely instead on the accuracy of their metrics and strength of their standard operating procedures. Every assay they use has already gone through development and validation. Furthermore, for any given assay, there are metrics available about what to expect for each step in the sequence and each instrument used. As long as the metrics come out they way they expect, these labs reason, their tests are accurate.

The Pros of Not Running Controls

- **It's easy.**
- **It doesn't "waste" space by adding controls to a run.** Space comes at a premium in any NGS run. To some labs, giving it up to add positive or negative controls means reducing throughput and possibly sacrificing reimbursement revenues.
- **It saves money.** NGS assays are expensive enough.

The Cons of Not Running Controls

- **Genomics tests are complex.** Next-generation sequencing tests comprise many sub-components, reagents, and systems — often from multiple vendors. If any part of a test begins to fail, it could impact the performance of the whole test. Without a rigorous incoming reagent quality control and acceptance program, you may miss underperforming reagents until it's too late and a major failure occurs.
- **It can take longer to troubleshoot, isolate, and resolve a problem** without the valuable data provided by a control run alongside patient specimens.
- **You can't track performance variations over longer periods of time**, and not doing so is risky (and may violate existing, documented operating procedures). A component or reagent may slowly cause subtle shifts in test performance over time.
- **You don't know what you don't know.** Some of the conditions you test for at your NGS laboratory are extremely rare. If you're just watching the metrics and not running controls, you may miss one. For example, according to statistics, Edwards syndrome (or trisomy 18) occurs one per 5,000 births. If you haven't had any results for Edwards syndrome lately, are you sure it's due to its rarity and not one of the many possible points of failure in the testing process?

Our final analysis: While it may save your lab some money, time, and space, not running controls is a risky decision that may end up costing you much more in the long run.

2. Developing Your Own (Homebrew) Reference Materials

Testing the accuracy of a process by comparing it to positive and negative controls is not only a tried-and-true stalwart of diagnostic testing, but the scientific method itself. Your known positive controls ensure your test is detecting what it's supposed to detect. If a known negative comes up positive, then you know you've got big problems — specifically, contamination.

> ***"By running a truth set of reference materials with known variants and allelic frequencies alongside patient samples, you can monitor daily, weekly, and monthly variations to assay performance, catching assay drift before it causes costly errors in test results."***
>
> ***— Dale Yuzuki, "Worried About Assay Performance Drift In Your Clinical NGS Assay? Avoid These 2 Common Mistakes"***

If you're committed to running controls, the questions become, then, where do you get the material for your controls? And what constitutes an effective control?

To answer the first question, some labs choose to create their own known-positive samples by growing their own cell lines or using remnant patient samples.

The Pros of Developing Your Own Reference Materials

- **It's more affordable than purchasing the materials from a vendor.** If your lab team already knows how to make the DNA on their own, you may find yourself thinking, "Why do we need to buy this when we can make it?"
- **Your lab already has the material on hand** in the form of remnant patient samples.

The Cons of Developing Your Own Reference Materials

- **It's inefficient.** How much of your team's time and energy do you want to dedicate to growing your own cell lines? It can be a work-intensive process that pulls your technicians away from other, more high-priority tasks, such as test development and implementation. You must also work to ensure you have a consistent process for creating your reference materials. Will there be lot-to-lot consistency? What manufacturing QC procedures will you implement?
- **They are unlikely to have the variant complexity and breadth** to properly measure the performance of NGS-based tests, which are highly-multiplexed and can assess hundreds of gene regions and a wide variety of mutation types. In other words, your residual patient sample will be a known positive for a single mutation, while most NGS-based tests look for a number of different mutations at the same time.
- **Different mutations and mutation types are more susceptible to performance variation than others**, as pointed out in a recent study from a University of California San Diego team.
- **Your supply of residual material will be limited**, and made even more limited because you'll need the patient samples you do have for yearly testing and validation. You may not even have any specimens that contain some of the more rare or difficult-to-sequence mutations.
- **The reference materials themselves may introduce performance variations that can mask assay drift.** Along with your homebrew material and remnants, you'll need to practice proper lot control to prevent this from happening.

Our final analysis: Developing your own reference materials is a step in the right direction — at least you're running controls to ensure the accuracy of your assays. But your homebrew reference materials may not be fully up for the job, may be in short supply, and will dominate precious lab resources.

3. Purchasing Quality Control Materials from Commercial Vendors

Because of the challenges and risks inherent in making their own reference materials, some labs turn to commercial suppliers.

"All of the careful design that has been included to help us address some of the challenges we see has been paying off. This is because we now have some references — some control materials — that we can use for the validation of the assays. With these we can validate the chemistry, we can validate the pipeline, to say, 'This is what I'm supposed to be detecting.'"

— Andrea Ferreira-Gonzalez, Ph.D., Chair of the Division of Molecular Diagnostics in the Department of Pathology at the Virginia Commonwealth University Health System.

Sometimes it's better to focus on what you do best — developing, validating, and running clinical genomics tests — and letting the experts do what they do best — creating quality control reference materials.

The Pros of Purchasing Reference Materials

- **You have access to an unlimited supply, whenever you need it.** For example, one SeraCare customer has access to literally millions of remnant patient samples, yet it still chooses to purchase materials from SeraCare simply because they will never run out. And, because SeraCare provides tight lot-to-lot control, this customer has found our material performs identically to the patient samples, under the same conditions, each and every time.
- **Commercial reference materials will offer a broad selection of mutations and mutation types**, from chromosome aneuploidy to dozens of point mutations, indels, and structural variants.
- **They have a proven shelf-life stability** of two years when stored under the proper conditions.

The Cons of Purchasing Reference Materials

- **It may add costs to your laboratory**, costs for which you aren't budgeting now.
- **It may be difficult to choose which vendor's products are right for your lab's needs.**

Our final analysis: The most efficient and reliable way to procure quality control testing materials for your lab is to buy them from a commercial vendor. Whatever additional costs you incur in the short term, you'll likely make up for in the long run due to increased accuracy, decreased downtime, and a sterling reputation for your lab.

3 Reasons to Choose SeraCare

In the previous section, we compared the three most common approaches lab directors use for monitoring the performance of their assays. We came to the conclusion that purchasing quality control reference materials from a commercial vendor is by far the most effective, most reliable strategy. It is the only way to be confident in your NGS lab's overall quality control performance and that the results you provide patients and clinicians are accurate 100 percent of the time.

If you've decided on using a commercial vendor for supplying your reference material, you'll find you have a number of vendors from which to choose. For the rest of this guide, we'll explain why we believe our company, SeraCare, stands head and shoulders above the competition.

There are three main reasons lab directors and pathologists choose to use our reference materials as the foundation of their quality control programs:

1. Our Products Are Highly-Multiplexed

Highly-multiplexed assays such as next-generation sequencing require highly-multiplexed QC products that can assess performance across the whole spectrum of variant types and variant frequencies. Our reference materials do just that. They contain a wide breadth of critically important mutations and mutation types for many disease areas.

An example:

Our Tumor Mutation DNA Mix v2 contains many mutation types including single nucleotide variants (SNVs), small and large insertions, and structural variations.

How are we able to produce reference materials with so many variant types and frequencies? With our unique technical approach: We combine highly-multiplexed biosynthetic constructs in a well-characterized genomic DNA background. These mutations are precisely titrated using digital PCR and results are reported for every lot.

2. Our Products Provide Patient-Like Ground Truth References

One of the concerns we hear most frequently from lab directors is about how "patient-like" our material is. That is, given the synthetic derivation of our products, can they truly be counted on to replicate samples that come from actual patients? To ensure run-to-run performance and delivery of the correct diagnostic results, lab directors want to get as close to the "ground truth" as possible.

Our many formats (purified RNA and DNA, FFPE cell lines, and cfDNA in plasma-like matrices) ensure labs have many options when assessing and trending assay over time. Much of our time and efforts go into our collaboration on developing the best NGS QC tools a lab can use to answer their specific questions--be it for assessing the robustness of the extraction and sequencing or if only to monitor the sequencing and bioinformatics performance. These materials are expert-designed and will enable labs to collect far more data with much lower costs.

> ***"Overall, results were indistinguishable from patient-derived data with variants being detected at or reasonably close to the targeted allelic fraction ratios."***
>
> ***— Dr. Birgit Funke, of Harvard Partners Healthcare, in a study of SeraCare's multiplex reference materials, published in the Journal of Molecular Diagnostics.***

3. Our Reference Materials Are Compatible With Our Laboratory QC Tracking Software

As we noted at the beginning of this guide, a truly robust quality control program includes two elements: running controls to monitor assay performance and tracking metrics. By tracking metrics proactively and at a granular level, you can catch problems before they get out of hand and their effect magnifies over an entire testing procedure.

Read more about the importance of tracking metrics in our recent paper, "2 Tools For Overcoming Your Clinical Lab's Toughest Quality Control Challenges."

But keeping track of all those metrics in a way that is both easily accessible and fully comprehensive is a tall order using hard copies and Excel spreadsheets (which lack key functionality) and laboratory information management systems (which are intimidating software packages, to say the least). That's where our iQ NGS QC Management Software comes in.

The iQ NGS QC Management Software is a powerful tool that simply and efficiently helps labs collect and track all their relevant NGS quality control metrics. By tracking all your QC metrics and run results over time and keeping them in a single location, you will:

- Save time and money preparing for CAP/CLIA inspections and audits.
- Be able to recall metrics, at a moment's notice, to help with troubleshooting and managing day-to-day variation in an assay.

We are ISO 13485-certified manufacturers and our products are rigorously quality and lot controlled.

You can rely on our materials as unchanging truth sets that will permit your lab to track performance for months and even years.

Get Started With SeraCare

As a lab director or pathologist with a clinical genomics laboratory, you've got enough on your mind. We hope the information in this guide has helped put to rest some of your worries about providing accurate results and catching errors early.

If you're ready to find out for yourself how SeraCare's highly-multiplexed reference material can help your lab, contact us today. You may also call us toll-free at 800-676-1881. We're looking forward to helping you and your entire laboratory team find the peace of mind only a reliable quality control program can bring.