BEFORE EDITING

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Buyer Persona 1 (Lab QC Operations), Question 4

Blog Post Title: Detecting slight differences in a clinical laboratory test over time

Subtitle: You need to pick up nuanced differences in your test performance to ensure against potential causes of assay failure. Do you have the tools needed to do the job?

For any clinical test, assay Limit of Detection (“LoD”) for sensitivity measurements is a key measurement metric, and any degradation of assay performance will make itself apparent here. Diagnostic test providers provide a ‘high positive’ control in their assay kit, and make a claim around sensitivity; however materials around the limit of detection are commonly not provided. A diagnostic laboratory can then be led to have a false sense of security as the ‘high positive’ indicates the assay is working for detection qualitatively, but not quantitatively.

A sentinel early-warning system

In the early days of coal mining before the development of sophisticated sensors for carbon monoxide poisoning, a caged canary was placed as a sentinel species <https://en.wikipedia.org/wiki/Sentinel_species#Historical_examples> to indicate danger, first proposed in 1913. The bird’s rapid breathing rate and high metabolism compared to humans gave sufficient time for the miners to seek safety - as long as that animal was regularly referred to.

In a clinical test environment reference material at or near the LoD will serve the same purpose as an early-warning mechanism for assay drift or a sub-optimal assay condition. Yet many laboratories do not have any kind of mechanism in-place to alert that there is a problem with an assay. The positive control, measuring a 50% allele frequency for the mutation of interest, is showing positive, and an erroneous assumption is made - that the 5% sensitivity measured during a validation phase of an assay has remained the same over time.

A convenient and reliable source of LoD reference standards

One method of obtaining reference standards that are close to the limit of detection is for a laboratory technician to make their own. A cell line with a heterozygous mutation (50% allele frequency of the mutation in question) can be diluted with a wild-type cell line ten-fold to obtain a 5% allele frequency, and that material can be subsequently be used.

However there are several issues with the ‘do it yourself’ method: first is the time, effort and reagents required to grow cells; second is the time, effort and reagents required to characterize them; third is the time, effort and reagents to insure stability over time; fourth is the time, effort and reagents needed to make sure the cell lines in question have stable copy number of the mutation of interest.

A commercial source of reference materials (such as from SeraCare) <https://www.seracare.com/products/controls-and-reference-materials/ngs-reference-materials-ruo/> solves these problems at once. From digital PCR characterization of the variants of interest to ISO and cGMP qualified facilities and 30 years of reference standard manufacturing experience a trusted supplier to the diagnostic industry makes your choice an easy one.

Software tools to keep QC data organized

Having reference materials near the LoD is the first important step toward efficient laboratory quality operations; however this data needs to be organized in such a way as to track this data across time, as well as track this data across users, instruments, and reagent lots. In a Laboratory Information

Management Systems (LIMS) context data scattered throughout SQL tables need specialized effort to extract this data; in a smaller laboratory operation without LIMS this data is scattered across shared spreadsheets and sometimes individual computers.

An elegant solution to this data management problem is the SeraCare iQ NGS QC Management Software <https://www.seracare.com/technology/iq-ngs-qc-management-software/> where its flexibility and power enable any clinical genomics laboratory to quickly track limit of detection and assay performance in an efficient and user-friendly manner.

How well-equipped are you to detect slight assay difference over time? Do you have the proper reagents and software in-place as a sentinel warning system?

AFTER EDITING

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2 Steps to Detect Slight Differences in Clinical Laboratory Tests Over Time

Nuanced differences in your test performance can cause assay failure. Do you have the tools to detect them?

Like a canary in a coal mine, limit of detection (“LoD”) reference materials can serve as an early warning system against degradation of assay performance for any clinical test. By providing a lower limit for the detection of a mutation, LoD reference materials prevent assay drift and sub-optimal assay condition — and therefore, costly errors in test results.

For two reasons, unfortunately, LoD reference materials are not that easy to come by:

1. Diagnostic test providers often do not provide LoD materials.
2. A do-it-yourself solution can take up time and resources you don’t have.

No LoD Materials in Assay Kits

Diagnostic test providers usually include a “high positive” control in their assay kits and make a claim about their sensitivity. However, they commonly don’t provide materials around the limit of detection — this, despite the fact that LoD is a key metric for measuring sensitivity.

This can give your diagnostic laboratory a false sense of security; the “high positive” indicates the assay is working for detection qualitatively, but not quantitatively.

Let’s say a positive control, measuring a 50 percent allele frequency for the mutation of interest, is showing positive. This can lead you to make an erroneous assumption: that the 5 percent sensitivity you measured during the validation phase of the assay has remained the same over time.

Why Not Make Your Own LoD Reference Material?

One way to get reference standards that are close to the limit of detection is to have a laboratory technician make their own.

The process is fairly straightforward, in theory: The technician can dilute a cell line with a heterozygous mutation (50 percent allele frequency of the mutation in question) with a wild-type

cell line ten-fold to obtain a 5 percent allele frequency. Your lab can use the result as reference material.

However, there are several problems with the “do-it-yourself” method. Mainly, it eats up precious lab resources:

- The time, effort, and reagents required to grow cells.
- The time, effort, and reagents required to characterize them.
- The time, effort, and reagents to ensure stability over time.
- The time, effort, and reagents needed to make sure the cell lines in question have a stable copy number of the mutation of interest.

Two Steps to Obtain and Manage LoD Reference Materials

Rather than go without and risk assay failure, or make your own and waste valuable lab resources, here is a two-step process for finding and using LoD reference materials:

Step 1: Find a Commercial Source of Reference Materials

A commercial source of reference materials (such as SeraCare) solves these problems at once. Look for a trusted supplier to the diagnostic industry.

SeraCare offers digital PCR characterization of the variants of interest, ISO- and cGMP-qualified facilities, and has 30 years of reference standard manufacturing experience — making your choice an easy one.

Step 2: Use Software Tools to Keep Your QC Data Organized

Once you have reference materials near the LoD, you’re well on your way toward efficient laboratory quality operations. However, you still need to organize this data so you can track it across time, users, instruments, and reagent lots.

If you use a Laboratory Information Management System (LIMS), you know that it takes specialized effort to extract this data scattered throughout SQL tables. In smaller laboratory operations without a LIMS, this data is scattered across shared spreadsheets, and sometimes, individual computers.

The SeraCare iQ NGS QC Management Software is an elegant solution to this data management problem. Its flexibility and power enable any clinical genomics laboratory to quickly track limit of detection and assay performance in an efficient and user-friendly manner.

How well-equipped are you to detect slight assay difference over time? Do you have the proper reagents and software in place as a sentinel warning system? Contact us and we’ll talk about it.