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VALIDATING THE SIMOA TECHNOLOGY

Frontage is the first and only laboratory to validate Quanterix' Simoa HD1 Analyzer for performing immunoassays.

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TABLE OF CONTENTS

VALIDATING THE SIMOA TECHNOLOGY

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Introduction

Most of the advanced instruments used in bioanalysis—such as Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), and Liquid Chromatography-Mass Spectrometry (LC-MS) for example—are automated. Thus, they are controlled by complex computer systems, and the information they generate is stored electronically. If these systems are to be used in regulated research studies in the US, EU, and Japan, the instruments themselves must be qualified and their supporting software and processes must be validated as meeting regulatory requirements.

Frontage Labs, having installed Quanterix' fully automated Simoa™ HD-1 system for performing immunoassays, has now successfully validated its use. Frontage is the first and only laboratory to do so, and is therefore the only laboratory capable of analyzing biomolecules with Simoa technology to support drug development in both pre-clinical and clinical phases. Because the Simoa system is ultra-sensitive, it is capable of detecting and quantifying biomolecules that would otherwise be difficult or impossible to measure, making it well suited to pharmacokinetic (PK), anti-drug antibody (ADA), and biomarker analysis.

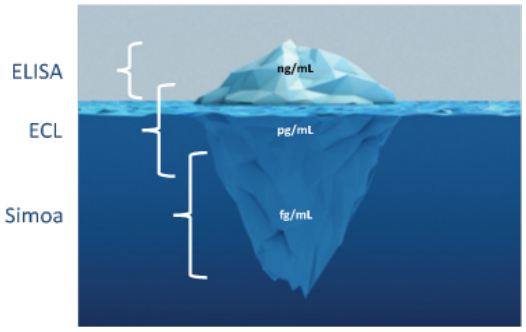
***FIRST CRO TO VALIDATE** SIMOA HD1 ANALYZER FOR PERFORMING IMMUNOASSAYS*

Here we describe the ground breaking Simoa system, discuss its benefits in biomolecular analysis, and review the steps that Frontage has undertaken to ensure that it meets current regulatory requirements.

Simoa Technology: Sensitivity Equivalent to PCR

The Simoa HD-1 system (the name is derived from single-molecule array) is ground-breaking in that it is up to 1,000 times more sensitive than traditional ELISA analog readout systems. It offers sensitivity equivalent to polymerase chain reaction (PCR), at a fraction of the cost. In fact, it can detect and quantify biomolecules previously difficult or impossible to measure (See Figure 1.) and has applications in PK, ADA, and biomarker analysis.

Figure 1: Measuring What Could Not Be Seen Before



Unlike analyses that rely on traditional ELISA and electrogenerated chemiluminescence (ECL) and which are limited to the picomolar range and above, the Simoa methodology is capable of measuring proteins at femtomolar concentrations.

Today, although the human proteome contains over 2,500 secreted proteins, only approximately 100 therapeutic proteins have FDA approval and are in use in medical practice today. Most of the "missing" proteins are simply below the detection limit of conventional immunoassays. The more sensitive measures possible with Simoa will likely open new applications for addressing unmet needs with new treatment regimens. It is possible that Simoa will support the earlier detection of cancer and infectious diseases and the identification of new biomarkers used in vitro and companion diagnostics.

The Simoa system also offers efficiencies in the laboratory. It can multiplex, running up to a 10-plex on a variety of analyte panels. This preserves samples, saves costs on consumables, and dramatically increases throughput. The equipment is also completely automated; it prepares samples (does the mixing, washing, and incubation) as well as provides the readout.

VALIDATING THE SIMOA TECHNOLOGY

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How The Simoa Works

Quanterix's Simoa technology is used to measure proteins in a variety of different matrices, including serum, plasma, cerebrospinal fluid, urine, and cell extracts.

Simoa takes advantage of the natural, selective attraction of proteins to enzyme-linked immunosorbent assays (ELISA) that produce detectable fluorophores. Traditional ELISA-readout systems, however, are analog and use conventional plate readers to measure the signals generated by molecules. Reaction volumes are relatively large; it takes millions of molecules generating tens of millions of fluorophores before an optical signal can be detected. In fact, this methodology can only detect signals at the picomolar range and above.

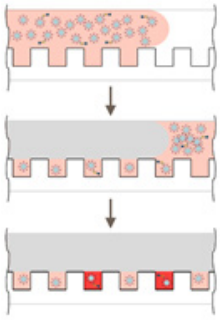
***1,000 TIMES MORE SENSITIVE** THAN TRADITIONAL ELISA ANALOG SYSTEMS*

In contrast, Simoa is a digital ELISA system that isolates individual molecules; it can detect the presence or absence of a signal, molecule by molecule.

The first step in the Simoa analysis is to concentrate a dilute solution of molecules with hundreds of thousands of paramagnetic beads, each with thousands of attachment sites. As the molecules collide with the beads, antibody capture agents attach to the surface of the beads—a process that takes less than a minute given that there are many more beads than protein targets. In this way, each bead that has captured a single protein molecule is labelled with an enzyme, and beads that do not bind to a molecule remain label free.

Next, the beads are washed to remove any non-specifically bound proteins. The beads are then loaded into arrays of femtoliter-sized reaction chambers, or wells, in the presence of a substrate. Each well accepts a single bead-immunocomplex, and the wells are then sealed. (See Figure 2.)

Figure 2: Simoa HD-1 Analyzer Femtoliter-Sized Wells



The isolation of each bead-immunocomplex allows the enzyme bound to the capture antibody to produce sufficient fluorescence in each well to be detectable, even when just a single molecule is present.

In the wells in which a target molecule has been captured, the enzyme label will produce sufficient fluorescence to be detectable. Thus an "on" well (one that contains a target molecule) can be distinguished digitally from an "off" well, and the precise protein concentration in the sample is a factor of the number of "on" wells in proportion to the total number of wells containing beads.

Applicable Regulations and System Requirements

In the US, the use of software in regulated analyses must conform to Good Laboratory Practices (GLP) and Good Clinical Practices (GCP). The applicable regulatory guidance is detailed in the Code of Federal Regulations (CFR) Title 21, Parts 11 and 58, as well as in Good Automated Manufacturing Practice (GAMP 5) guidelines, and Good Clinical Practice guidelines, Computerized Systems Used in Clinical Investigations, 2007.

21 CFR Part 11 deserves special mention. It specifies that all systems governing any clinical process should be validated to ensure that electronic records are trustworthy, reliable, and compatible with FDA procedures. With regard to system validation, the regulation states:

Persons who used closed systems to create, modify, maintain, or transmit electronic records shall employ procedures and controls designed to ensure the authenticity, integrity, and, when appropriate, the confidentiality of electronic records. Such procedures and controls shall include the validation of systems to ensure accuracy, reliability, consistent intended performance, and the ability to discern invalid or altered records.

The Code also requires that "Computer systems (including hardware and software), controls, and attendant documentation maintained under this part shall be readily available for, and subject to, FDA inspection."

For systems used in the clinical development and manufacture of pharmaceutical products to be authentic, reliable, accurate, and secure, they must have specific capabilities and safeguards in the following areas:

- Records Generation: Electronic records created, modified, maintained, and archived by the system must be accurate and readily retrievable. Procedures must be in place for making revisions and controlling changes to data.
- Account Management: System access must be limited to authorized individuals via user accounts controlled by passwords and unique user IDs. Authority checks must ensure that only authorized individuals can use the system.

VALIDATING THE SIMOA TECHNOLOGY

Frontage is the first and only laboratory to validate Quanterix' Simoa HD1 Analyzer for performing immunoassays.

- Configurable Roles: The software must acknowledge and support various user roles, each having permission to perform only specific functions and access certain information.
- Audit Trail: The system must record the date and time of operator entries and actions that create, modify, or delete records. This allows study events (such as user access, security setting changes, error messages, and sample processing steps) to be reconstructed and modifications to be tracked.
- Data Security: Protections must be in place to prevent electronic records from being deleted either intentionally or accidentally.
- Data Backup and Archival: Steps must be taken to ensure that system data can be restored in the event of a crash and that finalized data are protected and can be retrieved in the future.

Validating software to comply with regulations, then, is a matter of testing and documenting its performance—and the processes that surround it—against these requirements.

Best Practices in System Validation

Frontage Labs is keenly aware that modern laboratory instrumentation can be a "game changer" both for our business and that of sponsors. However, the acquisition of advanced instruments alone is not sufficient to further medical science and sponsors' business goals. As explained above, laboratory equipment—such as the Simoa HD-1 analyzer—must be validated to be used in a regulated environment, and this requires the expertise of a strong computer software validation team.

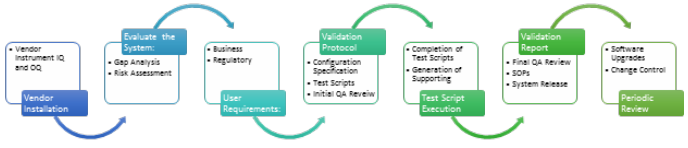
Over the years, Frontage's specialists in software validation have adhered to industry best practices and adopted several guiding principles to ensure that the process is both efficient and leads to full compliance. These include:

- Validating for both business and regulatory requirements. The validation exercise should not only verify conformance to regulatory standards, but also confirm that the system (and all attendant processes) will deliver the performance that clients expect.
- Relying on automated steps. Whenever possible, the validation steps should be automated to improve both efficiency and reproducibility.
- Working as a multi-disciplinary team. Ideally, the validation exercise should include a validation professional, a technical scientist who understands the software application, a quality assurance representative, and management. It is also important to work closely with the instrument vendor to more fully understand the software and discuss any questions or concerns.
- Treating validation as an ongoing concern. Maintaining a validated state requires a proactive approach and re-visiting the process anytime that the regulations change or that the software is updated.
- Staying abreast of new developments. It is important to stay current with the best practices in software validation and the most current recommendations put forth at national meetings and workshops, as well as to stay attuned to regulatory updates.

The Frontage Labs Validation Process in Brief

Although the process for validating software is somewhat standardized (See Figure 3) across industries and applications, it is nonetheless a comprehensive and detailed exercise that requires the knowledge of a validation professional working in concert with both a technical scientist who is intimately familiar with how the instrument will be used and with a representative from the instrument vendor.

Figure 3: Standard Software Validation Workflow Diagram



The following are highlights of the Frontage process, which began after Quanterix installed the platform and performed both an Instrument Qualification (IQ) and Operational Qualification (OQ) to confirm and document that the instrument is working according to the manufacturer specifications:

- The Frontage team evaluated the Simoa system, performing a gap analysis to compare the system functionality with the 21 CFR Part II requirements. They then completed a risk assessment which helped define the extent and focus of the validation. (The more critical the records, the more testing would be required.)
- For this project, it was critical to have an interface for transferring data securely from the Simoa system into our Watson Laboratory Information Management System™ (LIMS) from Thermo Scientific. Quanterix and Frontage worked together to meet this requirement, and as a result, Quanterix released a service pack that included an enhancement to facilitate the transfer process.
- The next step was to define all the user requirements formally,

VALIDATING THE SIMOA TECHNOLOGY

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both from a business and regulatory perspective. These requirements defined specifically what needed to be tested.

- At this point, the team prepared the Validation Protocol which detailed the validation strategy, the roles and responsibilities of all concerned, and the deliverables. They specified how the system needed to be configured (for example, defining which functional or security permissions would be assigned to the different user roles). They also wrote test scripts against the business and regulatory requirements. (Test scripts are instructions to be performed on the system to ensure that it performs as intended.) The Validation Protocol was then reviewed by Quality Assurance.
- Once the Validation Protocol was approved, the next step was to execute the test scripts through user acceptance testing. This involved testing the entire system in its environment against the defined requirements. Each of the user requirements was confirmed as being met in the test scripts, and the results were reviewed by Quality Assurance.
- Once Standard Operating Procedures (SOPs) governing system use, administration, calibration, and maintenance were in place, the final Validation Report was issued, summarizing the validation activities and deliverables.
- The system was then "released," meaning that it has been officially validated to perform as intended and is suitable for regulated production use.

Given that validation is not a static condition, but an ongoing process, Frontage will be performing periodic reviews of the system. These will be performed at least annually and whenever the software is upgraded. Any changes to the system will follow the change control procedures defined in the SOPs.

Our validation documentation is available for inspection by authorities and interested sponsors. It explains our validation plan and reports all results, beginning with the IQ and OQ steps all the way through to the creation of our SOPs.

Conclusion

Frontage Labs is proud to be the first and only Contract Research Organization (CRO) to validate use of the Simoa HD-1 analyzer for quantitative analyses in a regulated environment. The ultra-sensitive technology gives researchers a cost-effective way to measure previously undetectable target molecules, and because it is validated within Frontage labs, the system can be used in regulated pre-clinical and clinical studies. The Simoa HD-1 analyzer is available for use in PK, ADA, and biomarker analyses that will be submitted to regulators and may be instrumental in identifying new drug targets and biological pathways.

We invite all interested sponsors to review our Validation Summary Report and all the accompanying documentation. Please contact us at sales@frontagelab.com for more information.

Frontage Laboratories, Inc. is a CRO providing integrated, scientifically-driven research, analytical and development services throughout the drug discovery and development process to enable biopharmaceutical companies to achieve their drug development goals. We offer our clients comprehensive services in analytical testing and formulation development, drug metabolism and pharmacokinetics (DMPK), bioanalysis, preclinical safety and toxicology and early phase clinical studies. We have enabled many innovator, generic and consumer health companies of all sizes to file IND, NDA, ANDA, BLA and 505(b)(2) submissions in global markets allowing for successful development of important therapies and products for patients. We have successfully assisted clients to advance hundreds of molecules through development to commercial launch in global markets. We are committed to providing rigorous scientific expertise to ensure the highest quality and compliance.