RULES BASED MEDICINE
a Q² Solutions Company

Operational Procedures

Introduction

Rules-Based Medicine (RBM), a Q² Solutions Company, has spent over 20 years developing and optimizing multiplex immunoassays based on the Luminex xMAP® technology. More recently, we have applied these optimization tools to the Quanterix Simoa™ platform to provide measurement of biomarkers previously difficult or impossible to measure. The RBM platform combines the sensitivity and dynamic range of Luminex and Simoa microsphere-based immunoassays with the precision and dependability of automated liquid handling systems, advanced quality monitoring, validated data reporting processes, and a highly trained and dedicated staff.

Together these features set RBM apart from other biomarker testing laboratories by providing the highest quality, cost-effective approach to quantifying biomarkers from a variety of biological samples.

Quality and accreditation

We've designed our laboratory and component systems to deliver the highest quality data and to comply with the highest industry standards. RBM maintains a CLIA certificate of accreditation, performs internal audits, and participates in a proficiency-testing program comprised of external and internal assessments to ensure quality throughout the organization. We invite our customers to visit and audit our facility to review our high quality operations, procedures, and processes.

Our policies are in compliance with the applicable portions of the following regulations

- Good Laboratory Practices (21 CFR Part 58)
- Quality System Regulation (21 CFR Part 820)
- Electronic Signatures and Records (21 CFR Part 11)
- Clinical Laboratory Improvement Amendments [CLIA] (42 CFR Part 493)
- Good Clinical Practice (ICH E6)

Customer focused

A Project Management (PM) team member regularly interacts with each customer to fully understand expectations, timelines, and study requirements. The project manager then facilitates the execution of all testing activities for the customer and ensures that all requirements are documented and communicated to RBM's research and development and operational teams. Our Client Services team works closely with the PM team and addresses technical inquiries before study initiation and after data delivery, ensuring support throughout each project.

Driven by Standard Operating Procedures

Standard Operating Procedures (SOPs) direct all phases of our testing processes to ensure the highest quality, reproducible data. All SOPs, personnel training, working documents and forms are managed by MasterControl™ quality management software. Every stage of testing is outlined in an SOP with controlled documentation forms completed by laboratory personnel throughout the testing process.

1. Request to have the sample collection data included in the sample ID. Values can be separated by a comma or pipe. Note, since this value is what is used to track the sample through our systems, data reconciliation is not possible after analysis has begun.
2. Request to have the sample collection data included in separate columns on the lab report.

Option 1 is also available when utilizing the RBM Standard Vertical format.

HIGHLIGHTS

- Custom developed Laboratory Information Management Systems (LIMS) provides sample tracking, chain of custody, and data logging throughout the testing process.
- Sample and reagent storage locations are temperature controlled, monitored, and backed up with CO_2 and an on-site generator.
- Automated sample plate processing for efficient and reliable throughput.
- Four-step quality control procedure including instruments diagnostics, calibrator curves at front and back end of each reaction plate, three levels of controls run in duplicate, and sample review.

Sample receiving

Working with the customer to understand and meet their project needs is of absolute importance. Therefore, we require customers to provide any special processing requests and a sample manifest describing the incoming samples. We use this information to establish an electronic chain of custody that tracks the samples throughout the testing process. In addition, every sample received by RBM is assigned a unique 2D barcode that enables sample management system software to follow the sample through receiving, storage, preparation, testing, reporting and disposition.

Upon arrival of the samples at our facility, the ID on the received sample tube is verified against the sample ID manifest. In the event that there are discrepancies, we work closely with the customer to resolve all issues prior to releasing the samples for testing. Discrepancies can include: samples IDs that are inconsistent with the sample manifest, extra samples, missing samples, damaged vials, samples received thawed (in the case of samples shipped on dry ice). Once all discrepancies have been resolved, samples are released into the testing queue.

Testing your samples

At the scheduled time for sample testing, the samples are removed from the storage location and loaded into their assigned location on a 96 well amicrotiter plate. This process is verified using a computer-assisted monitoring process. Once loaded on the sample plate, original samples are returned to their assigned freezer storage location.

The sample plate is loaded onto one of RBM's advanced liquid handling robotic instruments that combines the samples with reagents to conduct the assay. The first step is to add microspheres that are conjugated to antibodies specific for the target analytes. Following an incubation, a cocktail of analyte specific detection antibodies is added to the microsphere mixture. Additional reagents are added to generate a signal, the microspheres are washed, and the plate is read on the appropriate instrument. All of our instruments are on a documented routine maintenance and calibration schedule.

Luminex measurement

The Luminex instruments operate similarly to a flow cytometer, using the principle of hydrodynamic focusing to pass microspheres, one at a time, along a path that is interrogated by two lasers *(Figure 1)*. The excitation beams measure the unique fluorescent signature of each microsphere and the amount of fluorescence generated is proportional to the analyte concentration in the sample. The Median Fluorescent Intensity (MFI) value of the measured microspheres is then calculated for each protein in the multiplexed assay.

Figure 1: Multiplex analysis by XMAP®

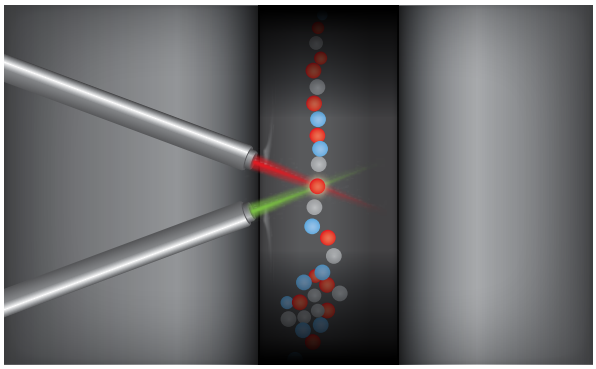
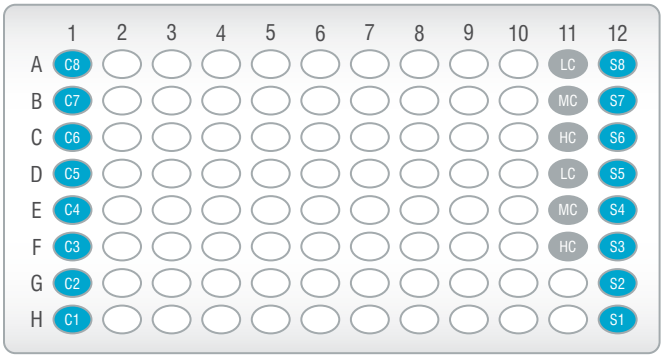


Figure 2: Standard curve fitting



Simoa measurement

The Simoa technology is also microsphere-based but utilizes a vastly decreased reaction volume compared to conventional immunological techniques. The signal generation volume in a Simoa assay is 2 billion times smaller, so a single target molecule in a well generates enough fluorophores to be measured using conventional fluorescence imaging. Microspheres, after the conventional immunoassay is complete, are mixed with enzyme substrate and loaded into a chip containing 216,000 individual femtoliter-sized wells. The digital signal is generated by counting the number of wells that are generating fluorophores. When concentrations of the target analyte reach levels above which digital calculations are meaningful, the system's algorithm converts to an analog measurement, ensuring accuracy across a wide dynamic range.

Quality control (QC) process

Assay results are processed through four quality control (QC) phases – instrument QC, standard curve QC, assay QC and sample QC. Only data that passes all four quality control phases is reported. We utilize internally developed software to analyze all of the data produced in an assay and if an anomaly is identified (e.g. absence of a robust signal, analyte concentrations that fall outside of the range of the standard curve, etc.) the results are checked and verified through repeat testing. Once the entire sample set has passed all of our quality control measures, a final report is produced for the customer.

Phase 1 – Instrument QC

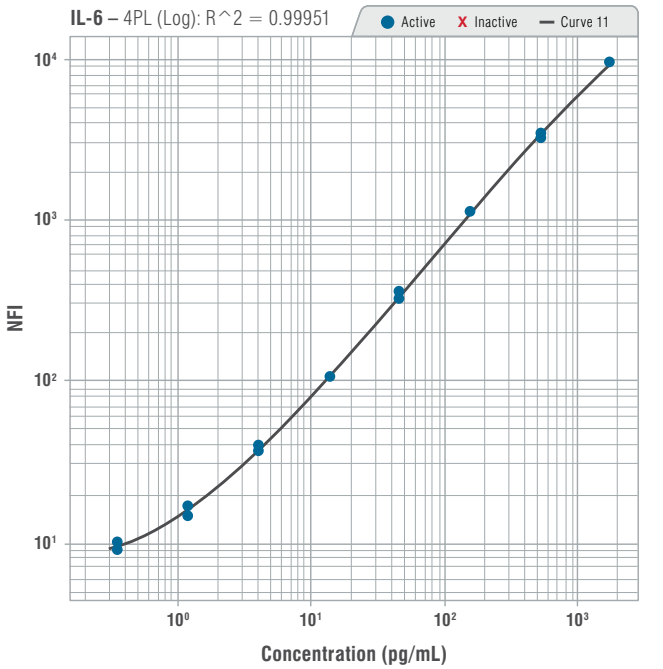
During the first phase of the data QC, our reporting personnel evaluate the instrument QC. This system flags data generated outside of temperature range specifications, data generated with a microsphere count of less than 50, and/or data generated with low laser power. Data with a microsphere count of less than 50 will be repeated.

Phase 2 – Standard curve QC

The next phase is the evaluation of the standard curve. For each multiplex, standards are placed in the first and last column of the reaction plate and processed alongside the samples. This flanked placement helps control for issues that may arise as the plate is processed because it allows us to readily detect potential inconsistencies between the duplicate standard values *(Figure 2)*.

This dual set of standard concentration values is fitted using our proprietary curve-fitting routines as seen in *Figure 3* (standard curve fitting image). Our algorithms use four and five parameter logistic fit equations to produce the best description of the standard values and are specifically tailored to include the "difficult-to-fit" points at the low and high ends of the curve. **Our curve fitting methods achieve R-squared values of > 0.99.**

Figure 3: Standard curve fitting

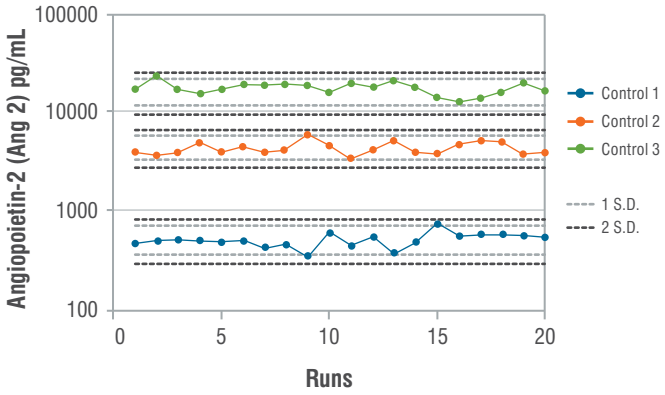


Phase 3 – Assay QC

The third phase in the QC process is assay QC. We use three levels of controls to cover the concentration range of the assay – low, medium, and high. The controls contain known values of the target analyte and are designed to mimic the sample matrix. We then evaluate the measured control results per level, per analyte, and per multiplex to ensure that the assay meets acceptance criteria.

Data from the controls are used to construct a Levey-Jennings chart for each assay. This enables us to follow the assay performance longitudinally, which provides information on assay trends *(Figure 4)*. Our assays have an exceptionally low intra-assay coefficient of variation – typically less than 10%.

Figure 4: Example Levey-Jennings chart for Angiopoietin-2 assay 3-level controls over 20 runs



We have adopted a set of standard clinical laboratory rules to evaluate control data in the context of an expected range. The expected range is based on data from a minimum of 30 replicate results generated over a minimum of three days with different operators and instruments considering statistical standard deviations creating a Levey-Jennings type analysis. These rules are a set of multi-rule QC decision criteria that are used to determine whether an assay is functioning as expected. The rules alert us to anomalies in individual control value levels, systemic problems among or within the controls, and potentially unwanted trends in the data. We use custom analysis of the control data from every run to automatically determine if control values are out of range. The controls must pass all QC criteria to be considered valid and we only report results from assays that meet this standard.

Phase 4 – Sample QC

In the last phase of the QC process, our Reporting team reviews sample annotations made during the testing process to determine whether or not the sample needs to undergo repeat testing. If no repeat testing is required, the data is then compiled into a report.

Reporting

Customer reports are generated using automated systems that compile the data from our secure database. The finalized report is auto-generated, auto-archived and auto-versioned. Our system records information about who has compiled or edited the file and logs any changes that are made, thus providing data traceability.

Data tracking and storage

All aspects of our sample testing process are carefully monitored and all data is tracked and archived. This effort starts with vendor qualification and assay manufacturing and extends throughout the operation until data delivery. All information, including Standard Operating Procedures, run specifications, sample plate templates, specified reagent lots, and QC acceptance worksheets, is tracked throughout the testing process.

Data is stored on our secure servers which are backed up daily. Weekly off-site backups provide long-term data security. All data is backed up for a minimum of 10 years.

Sample disposition

Samples are tracked throughout the entire testing process – from receipt to disposition – using our LIMS. RBM provides sample storage for a minimum of six weeks after delivery of the data report. RBM has Standard Operating Procedures for sample disposition and discards or returns the samples based upon the customer's request.

Conclusion

RBM's long history of immunoassay development and optimized, high-throughput operations allows us to provide customers with unmatched expertise and quality in the field of biomarker testing. We have developed automated systems and processes in a tightly controlled environment which produces highly reproducible results.

Contact us

Website: RBM.Q2LabSolutions.com

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