Upscaling of a Clinical Flow Cytometry Laboratory

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As the field of flow cytometry is constantly evolving, testing labs should strive for continuous improvement of operations and quality

Background

As flow cytometry is one of the go-to methods for fast and in-depth monitoring of immune cell populations at single cell level, clinical laboratories are observing an increase in assay complexity and number of samples to process. To scale up our operations in a qualitative and efficient manner, several aspects of the laboratory design were improved and are discussed below.

Antibody cocktail preparation

Manual steps in preparing antibody mixes remain a risk for introducing variation or errors in assays. One way to limit this is to purchase pre-made cocktails or dry or lyophilized reagent tubes. However, reagents from different vendors, multiple Brilliant Violet™ or tandem dyes, as well as drug-specific (and often non-commercial) reagents should be combined in laboratory developed tests for our clients' needs. To our knowledge, no custom commercial reagent cocktails can be obtained with acceptable shelf life, demonstrated inter-lot performance, in a cost-effective manner, given the often limited quantities required for each stage of a clinical trial.

A general standard operating procedure is used for preparation of antibody cocktails, working tube per tube and on ice throughout the process, with reagents and mixes protected from light wherever possible. To do so, reagents are arranged per tube in labelled, cooled and temperature-stable racks. This resulted in increased efficiency as well as improved consistency and accuracy of measurements.

For assays with high sample volume on a daily basis, antibody cocktails were also validated for stability. In our experience, stability was rarely over 7 days, likely due to interaction between fluorochromes, antibody conjugate instability or breakdown. Using amber vials to prevent photobleaching is a prerequisite.

Improved QC

Lot-to-lot validation is performed for all individual antibodies. However, antibody cocktail-to-cocktail validation remains challenging. Whereas testing on samples or quality control (QC) material is possible for less complex panels, many of our lab developed tests include markers which are only expressed in specific patient populations and/or in low frequencies. QC material expressing all markers is often not available.

QC for antibody cocktail can be performed on compensation beads to demonstrate that all antibodies were added, but reagent interactions can result in false positive signal. For these cocktails, QC is often only possible on patient samples and verification using acquisition sheet post-measurement is required.

Automated sample preparation

BD lyse wash assistant (LWA) is used for automated lysis and washing, freeing hands-on time for lab techs. Limitations include high cycle time, maximum volume of buffer addition in one step, restricted use of parallel buffers etc. Moreover, we observe important cell loss versus manual processing and therefore carefully consider each assay before running on LWA. Similar results were observed when testing Sysmex Rotolavit system.

Secondary tube labelling

Tube labelling for sample preparation is automatically driven by assay requests in LIS and independent of presence of sample. For each tube of each panel requested per sample, a label will be printed automatically by a tube label printer linked to the LIS.

Switch to BD FACSLyric™

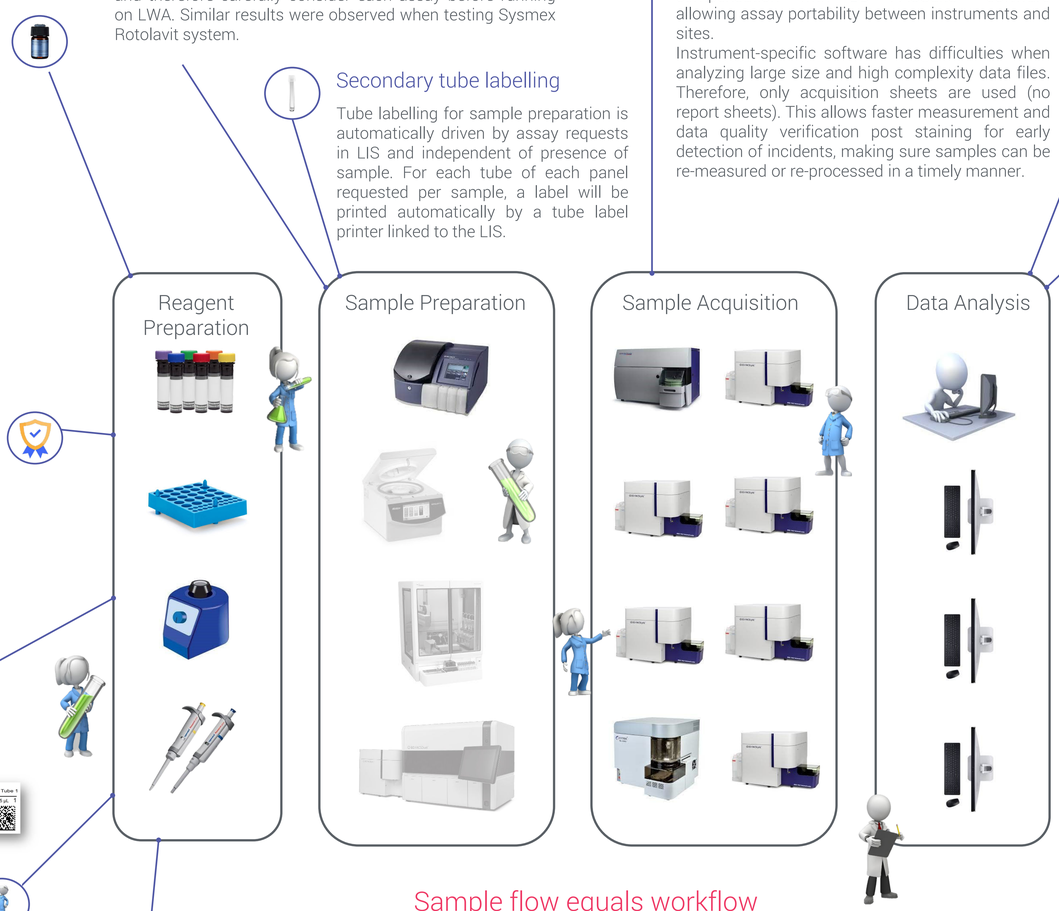
Switching from BD FACSCanto™ to BD FACSLyric™ resulted in faster sample acquisition with higher resolution of data. Improved instrument standardization with cytometer setup and QC software and controlling assay settings and compensation with BD FACSuite™ software allowing assay portability between instruments and sites.
Instrument-specific software has difficulties when analyzing large size and high complexity data files. Therefore, only acquisition sheets are used (no report sheets). This allows faster measurement and data quality verification post staining for early detection of incidents, making sure samples can be re-measured or re-processed in a timely manner.

Improved data analysis software

Instrument-specific analysis software is replaced by FCS Express™ data analysis software (De Novo), allowing user-friendly and customizable analysis templates and client-specific formatted reports. A network version of FCS Express™ software enables remote data analysis and allows data analysis being done independent from the instrument. This resulted in standardized, faster and more efficient data analysis and review regardless of location of scientists.

Globalization of middleware for online reporting to LIS

All instruments at all testing sites are connected to a fully validated global middleware for online reporting of results to the clinical trial management system (CTMS). Data reporting process does not involve manual entry steps from measurement on the instrument to analysis and reporting. Data reporting process includes a global repository where all raw data and sample reports are stored using automated filing and structuring of data.



Calculation sheets

For each cocktail we use calculation sheets. Number of samples is entered, and all calculations are automatically performed. Lab techs document addition of antibodies in all steps. Standardized cocktail labels mentioning date of preparation, tube number and assay name can be selected in a label printer.

Engaged staff

Engage staff in each improvement of lab operations, from concept to implementation. Act on feedback whenever possible. Provide continuous training. Motivated employees are key for higher quality and increased productivity.

Improved reagent management

Immediate-use reagents are available in well-organized fridges with designated locations per assay, located conveniently in the sample preparation area. Addition of a separate cooled storage room results in a clear and qualitative stock management and reduced waste. An automated min-max system was implemented for reagent ordering which allows to have minimum stock of reagents while maintaining continuity of lab operations.

Sample flow equals workflow

- A lean floor plan following the flow of a sample results in increased efficiency
- Semi-automated Data Cover Sheets used to trace all steps from sample preparation to reporting
- Automated Sample management
  - Fully automated system from original request in laboratory information system (LIS) before sample arrives in the lab to measurement on flow cytometer. Sample and assay-specific information is automatically completed on data cover sheet after measurement.
  - Remote accessible middleware allowing unlimited panel and reportable configurations, offline testing,... results in increased productivity.

Future considerations

Electronic signatures with the use of tablets
Whereas documenting of all steps in the lab often remains a manual process with subsequent paper storage of all documentation, switching to electronic documents using tablets with specific software for digital signatures can reduce paper whilst complying with regulatory requirements. Additionally, introduction of digital signatures for all steps from bench to reporting will increase traceability and minimize risk of errors.

Advances in commercial reagents and antibody cocktail preparation
Use of reagents from different vendors, inclusion of drug-specific reagents and combination of multiple Brilliant Violet™ or tandem dyes limits the use of commercially prepared cocktails in high-complexity flow testing. These challenges, combined with the need for pre-dilutions, also limit the implementation of current cocktail preparation instruments such as PS-10 (Sysmex), BD FACSDuet™ and CellMek (Beckman Coulter) for pipetting of complex panels. Mainly too many manual interventions are needed still. Ongoing efforts by vendors on both reagent side and automated reagent and sample preparation systems will help in increased efficiency, reproducibility and traceability of cocktailing and sample preparation steps. Simultaneously, advances are being made in the availability of commercial QC material, allowing verification of antibody cocktail preparation.

Automation and traceability of sample preparation and measurements
Given the variety of sample preparation methods and buffers used in complex assays, many systems are not yet capable to process samples as required for our clients' needs. Additionally, instruments capable of scanning sample barcodes are often only verifying, and not directing measurements.

Implementation of automated gating software and AI-assisted data analysis
As software becomes available and gains recognition for acceptability of use in global clinical trials, data analysis could be further improved by implementing software applications for more standardized and efficient data analysis and improved insight in clinical trial data.

Conclusion

All implemented optimizations greatly improved the quality, assay performance, traceability, productivity and turn-around-times of our lab operations. Novel developments in automation and traceability are constantly monitored and assessed. This way, we keep improving our complex, tailor-made operations for clinical trials. Continuous follow-up of advances in all steps of the process is how we maintain our high level of quality and productivity.

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Cerba Research can develop and validate customized flow cytometry panels for global clinical trials.
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