Process Improvement in Clinical Sample Testing with Quality Checks and Efficient Workflow for Global Clinical trials

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Abstract

Flow cytometry assay in clinical trials with multiparameter and high sample numbers require more efficient workflow. To ensure the robustness and accuracy of results, quality checks are integrated in the workflow. For antibody cocktail preparation, a dedicated "staining map" (Figure 1) for each panel is created in Excel to precisely calculate total antibody volume. To verify the antibody cocktail for the presence of all the antibodies, including humanized antibodies, Ultracomp eBeads™ Plus (Thermo Fisher Scientific) is used for staining with cocktail. Beads are acquired on the specific assay setting to ensure that the signal is optimal for all markers. Another process is implemented to avoid errors in setting up the assay in BD FACSLyric™ instrument. If one sample has multiple panels for testing, then opening all the assays manually in FACSuite™ could introduce errors. Middleware (Instrument Manager (IM) from Data Innovations) is implemented which communicates with lab information system (LIS) to identify specific tests ordered with a specific sample. This process improves efficiency and avoids any errors in selecting the assay. After the acquisition, one page acquisition template with multiple tubes provides additional verification for total events acquired, sample quality (poor lysis), and instrument fluidic issues (with time plot). Once sample is acquired, data analysis and review are structured to centralize for global studies. Data are reported automatically with FCS Express (De Novo Software) data analysis software and IM, removing manual data entry steps to reduce errors on data reporting (Figure 2). All these process improvements and quality checks significantly improve the workflow and makes the entire process more efficient in sample processing to data reporting for global clinical trials.

Staining Map

The staining map is an Excel file contains specific antibodies, catalog number and volume tested per panel. This is a useful tool in laboratory workflow, designed to minimize human errors during the staining process, particularly in complex assays involving multiple antibodies and fluorochromes. It provides a clear outline of the antibodies and fluorochromes for each panel, reducing the likelihood of errors and ensuring accurate results. The plug-and-play design allows technologist to input the number of samples required for staining, and the staining map automatically calculates the required volume of each antibody. This automation saves time and reduces the need for manual calculation and allowing them to focus on other critical aspects of the workflow.
Additionally, the staining map enhances documentation and accountability by requiring technologist to mark each antibody as it is added to the cocktail and sign off with his/her initials. This step ensures that each action is documented, providing a clear record of the staining process and enhancing traceability. By automating volume calculations, the staining map reduces the potential for calculation errors, ensuring the correct volumes are used consistently. This process ensures no antibodies are missing from the cocktail, which is crucial for the accuracy and reliability of the staining process.

# Samples	10	Assay Name	Performed by:	Sticker
# Blood / sample	100			

Sponsorspecific Antibodies:

Antibody	Lot #

Staining: all volumes in µL

	BV421	V500	BV605	FITC	PE	PerCP-Cy5.5	Pe-Cy7	AF647	AF700	APC-H7	Sample
		BM1			BSA	BM2	BM3		Viability	BM4	tube1
1		50			50	25	25		10	25	18.50
		BM1			CAR	BM2	BM3		Viability	BM4	tube2
2		50			50	25	25		10	25	18.50

Antibody	Fluorochrome	Cat#	New vial initial
BM2	PerCP-Cy5.5	V123	
BM3	Pe-Cy7	V456	
BM1	V500	V789	
BM4	APC-H7	V1011	
Viability	AF700	V1213	
BSA	NA	V1415	

*BM: Biological Marker

Fig. 1: Staining Map (Example page image in the Excel file)
Using the provided table, the technologist confirms the details of each antibody vial, including the marker name, fluorochrome, and catalog number. Before pipetting the appropriate volume as specified in the table, the technologist also indicates if new vials are opened by marking with initials.

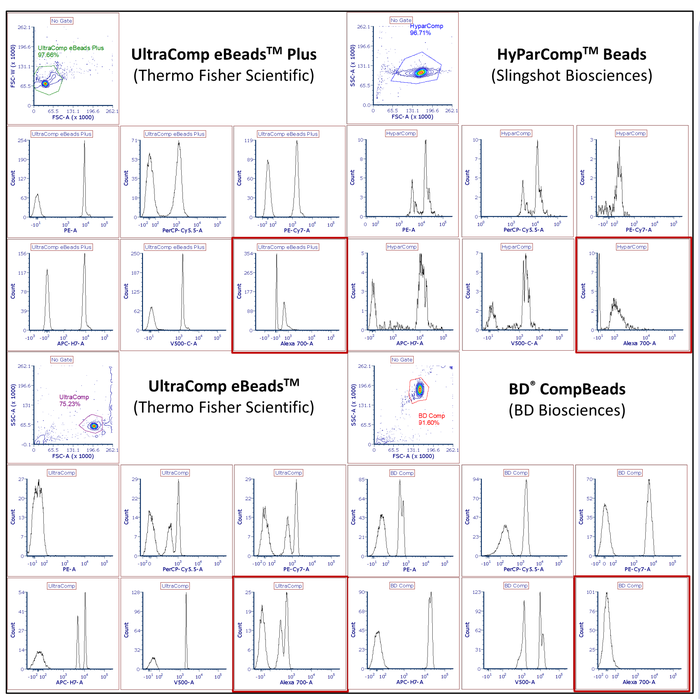


Fig. 3: Overview of Compensation Beads tested

Cocktail Verification

To minimize errors in the preparation of antibody cocktails, the technologist adheres to a standard operating procedure (SOP) and utilizes a staining map. Despite these precautions, errors can still occur, such as the omission of an antibody. To address this, we have implemented an antibody cocktail verification step using compensation beads.
The primary function of the compensation beads is to verify that the prepared cocktail contains all the markers specified in the panel. Additionally, these beads assist in detecting dim markers, such as PD-1, which are challenging to identify in cells.
We tested multiple compensation beads (Figure 3) to ensure the presence of all markers in the cocktail. The criteria for selecting the optimal bead included:

1. Good separation between negative and positive signals.
2. Ease of the staining procedure.
3. Post-staining stability of the beads.
4. Compatibility with multiple species, including humanized antibodies.

An overview of our findings is reported in Figure 3, which indicates that UltraComp eBeads™ Plus by Thermo Fisher Scientific provided the most accurate signal for all markers, including humanized antibodies present in the phycoerythrin (PE) fluorochrome. It should be noted that none of the tested compensation beads are suitable for detecting the presence of viability dyes (Alexa 700) highlighted in red in Figure 3. To identify viability dyes in the cocktail, Amine reactive beads should be utilized. This verification step has significantly enhanced the reliability and accuracy of our antibody cocktails in flow cytometry assays.

Data Reporting

The process of data reporting in a laboratory setting is challenging if there is no automated system, particularly in the naming or input of sample identifiers. Manual process can lead to significant issues, including incorrect test results, misdiagnosis, and potentially lead to compromised patient care. To reduce these risks, our workflow has been meticulously designed to minimize manual inputs and streamline the data reporting process.

Unique Identifiers and Test Coupling: Each sample received in the lab is assigned a unique identifier, which is then coupled with the requested tests. This step is crucial as it ensures that each sample is distinctly recognized and tracked throughout the testing process. The unique identifiers are uploaded into the LIS, which acts as the central repository of all sample-related information.

Integration with Middleware Systems: The LIS shares sample information with middleware system, BD FACSLink™/IM. Middleware system plays a pivotal role in laboratory operations by acting as intermediaries that facilitate communication between different laboratory instruments and the LIS. IM from Data Innovations is used to identify specific tests ordered for each sample. This integration is vital for improving efficiency and reducing the likelihood of errors, as it ensures that the correct assays are selected for each sample.

Technologist Workflow during Sample Acquisition: Technologist scans the barcode in the sample tube, which extracts the appropriate test(s) and opens the correct "worklist" (assay) in the BD FACSuite™ software. This automated process reduces the chances of human error, as the technologist is guided through each step with minimal manual intervention.

Centralized Data Analysis and Reporting: Once the sample is acquired on the BD FACSLyric™ instrument, FCS files are exported, and the data analysis and review processes are centralized. This process ensures consistency and accuracy in data interpretation. The use of FCS Express data analysis software and IM automates the reporting process, eliminating manual data entry steps that are prone to errors. The final, approved data is then sent to the LIS, where it becomes accessible to customers, allowing them to view the requested data for each sample.

Fig. 2: Sample Receipt to Data Reporting Workflow

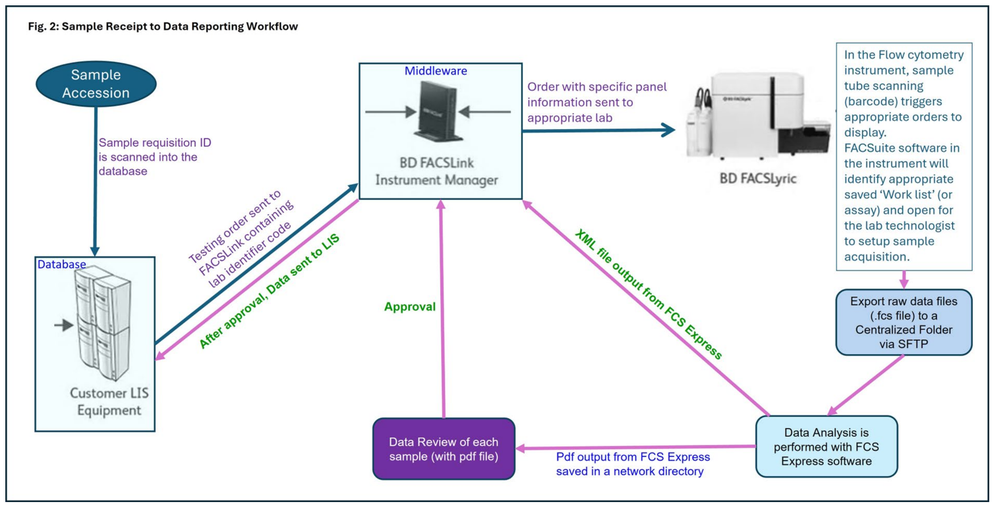
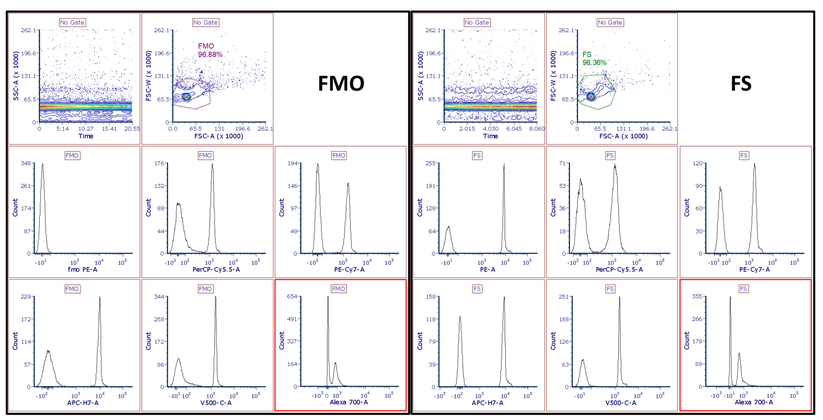


Fig. 4: Cocktail Verification
The presence or absence of an antibody is verified using the UltraComp eBeads™ Plus. A comparison of an FMO (fluorescence minus one) tube with a FS (full stain) tube is presented. The time plot indicates no aberration during acquisition. The PE plot shows a positive signal in the FS tube, indicating antibody presence, while the FMO tube shows only a negative signal as intended per panel. The viability dye, Alexa 700 (red plot) does not stain with UltraComp eBeads™ but can be determined using the amine reactive beads.



Species Compatibility

BD™ Comp Beads	HyparComp™ Beads
Mouse Rat Hamster	Mouse Rat Hamster
UltraComp eBeads™	**UltraComp eBeads™ Plus**
Mouse Rat Hamster	Mouse Rat Hamster Rabbit Human

Conclusion

- Streamlined workflow from sample to reception to data analysis minimizes errors in flow cytometry assays.
- UltraComp eBeads™ Plus ensures accurate signals for all markers, enhancing antibody cocktail reliability.
- Reduced manual inputs and advanced software solutions improve efficiency and reduce data reporting errors.
- More reliable test results lead to improved patient outcomes and greater confidence in lab operations.
- Faster turnaround time for data reporting benefit both the laboratory and the clients.

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Cerba Research can develop and validate customized flow cytometry panels for global clinical trials. Connect with our scientific team to learn how we can enhance your research and develop specific flow cytometry panels.