Flexibility in sample preparation for mixed laboratories running on a Single Sample Preparation device with alternatives in post-stain washing

Brescia: Marco Chiarini, Anna Galvagni, Viviana Giustini, Daniele Moratto, Duilio Brugnoni: Flow Cytometry Unit, Clinical Chemistry Laboratory, ASST Spedali Civili of Brescia, 25123 Brescia, Italy;
Ghent: Jana De Wolf, Annick Willems, Mattias Hofmans: Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium
BD: Ira Racoma, Lori Apoll, Lucia Testolin; Maureen Martin, Nicolas Bailly, Anne-Catherine Dolens, Manuele Ongari: BD Biosciences, San Jose, CA 95131, U.S.

NPM-1732

Introduction

Advanced sample preparation automation in flow cytometry laboratories utilizes on-board centrifugation to process samples, similar to bench processing. For laboratories running both Lyse-No-Wash and Lyse-Wash assays and without a system with on-board washing capabilities and/or using methods requiring system off-board procedure, a post-stain off-board wash processing might be advantageous for workflow purposes.

The BD FACSDuet™ Premium Sample Preparation System is a fully automated sample preparation device with on-board sample washing and on-board centrifugation and was used to assess a post-stain on-board wash method compared with two semi-automated / manual workflows with post-stain off-board wash.

Metrics measured were a) Total Process Time (TPT) for both fully automated workflows and workflows with post-stain off-board washes; and b) Hands-On Time (HOT) required during sample processing.

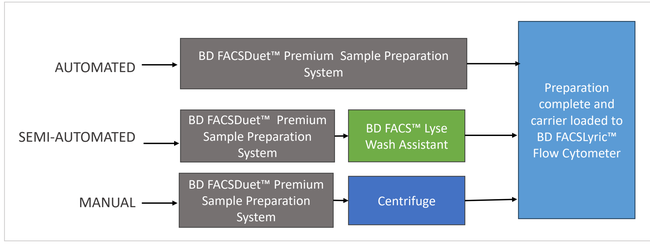
Method

Two sites participated in this study. They compared two post-stain off-board wash methods with a fully automated post-stain on-board wash one. *(FIGURE 1)*

In the specific, for all three (3) conditions, a BD FACSDuet™ Premium Sample Preparation System physically integrated with a BD FACSLyric™ Flow Cytometer for automated transfer and acquisition of stained and washed samples were used. Pre-washed off-board specimens (excluded from time measurements) and secondary tubes containing dried reagents were loaded on the BD FACSDuet™ Premium system. All scenarios were run with 16 secondary tubes.

1. The entire AUTOMATED process - comprising of specimen dispensing to secondary tube, reagent incubation, lysing addition and incubation, 3x post-stain washes - was performed on board of the BD FACSDuet™ Premium Sample Preparation System; stained samples were automatically transferred for acquisition on to the physically integrated BD FACSLyric™ Flow Cytometer.
2. The process from specimen dispensing to lysing was performed on board of the BD FACSDuet™ Premium Sample Preparation System, followed by off-board removal of stained and lysed samples for SEMI-AUTOMATED off-board washes on a BD FACS™ Lyse Wash Assistant (LWA), and loading of the off-board washed samples to the BD FACSLyric™ Flow Cytometer for acquisition. *(further details in TABLE 1)*
3. The process from specimen dispensing to lysing was performed on board of the BD FACSDuet™ Premium Sample Preparation System, followed by off-board removal of stained and lysed samples for MANUAL off-board washes on a laboratory centrifuge, and loading of the off-board washed samples to the BD FACSLyric™ Flow Cytometer for acquisition. *(further details in TABLE 2)*

FIGURE1: Workflows



Please note: TPT, HOT and Error prone tasks are highly dependent on the method used for preparation, etc. Meaning that these data may not be representative of what other labs may achieve . However, if the exportable preparation protocol option on the BD FACSDuet™ Premium Sample Preparation System is used, the automated portion of the preparation can be standardized within labs and across collaborating labs.

Analysis & Results

Samples were tested using the methods described in TABLES 1 & 2 in comparison to complete processing on the BD FACSDuet™ Premium Sample Preparation System.

- Process times started with "Run Worklist" to remove any variability attributed to the manual technique used by the laboratory in loading the BD FACSDuet™ Premium Sample Preparation System with specimen, secondary tubes, reagents.
- The fully automated process had neither post-stain manual intervention nor Hands-On time in the measured process being assessed *(TABLE 3)*

Comparisons at both sites demonstrated no difference between preparation with full BD FACSDuet™ Premium System sample processing with 0.0% HOT *(FIGURE 2 and TABLE 3)* and TPT *(FIGURE 3 and TABLE 4).*

Post-stain off-board wash using semi-automated BD FACS™ LWA showed 0.6% faster TPT between sites *(TABLE 4)*, with average 1.5% HOT *(TABLE 3).*

Post-stain off-board wash using traditional (manual) centrifugation had TPT 21.9% longer at Site 2 *(TABLE 4)* with average HOT across both sites of 30.3%. *(TABLE 3).*

Full automation with BD FACSDuet™ Premium Sample Preparation System might not decrease total process time but reduces hands-on-time and hands-on tasks, reducing the risk for errors.

FIGURE 2: Hands-On Time and Total process Time

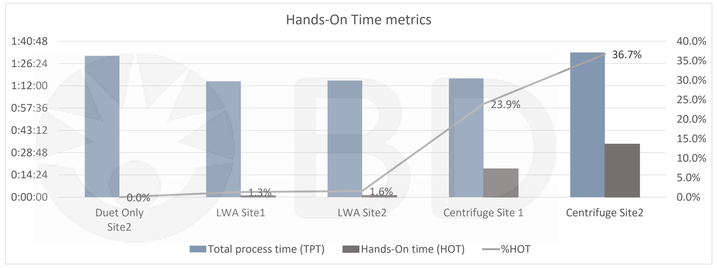


TABLE 3: Hands-On Time

Description	BD FACSDuet™ Premium System Only Site2	BD FACS™ LWA Site1	BD FACS™ LWA Site2	Lab Centrifuge Site 1	Lab Centrifuge Site2
Total process time (TPT)	1:31:21	1:14:53	1:15:21	1:16:46	1:33:33
Hands-On time (HOT)	0:00:00	0:01:00	0:01:12	0:18:21	0:34:19
%HOT	0.0%	1.3%	1.6%	23.9%	36.7%
%Difference HOT Site1 to Site2		-0.6%		-21.9%	
Ave HOT	0.0%	1.5%		30.3%	

Site 1: University Hospital Ghent – Belgium
Site 2: ASST degli Spedali Civili Brescia - Italy

TABLE 1: SEMI-AUTOMATED Post Stain method

BD FACSDuet™ Premium Sample Preparation System to BD FACS™ Lyse Wash Assistant	
Process on BD FACSDuet™ Premium Sample Preparation System	Process from specimen dispensing to lyse incubation Carrier was unloaded for post-stain off-board wash
Process for washing on BD FACS™ LWA (IVD protocol *WASH Only)*	Wash procedure on each sample tube uses 6,4ml BD® CellWASH
	Cells are pelleted in 10 sec at a precipitation G force of 461g, the buffer is added while the tube is spinning at a Wash G force of 350g at a flow rate of 800ul/sec..
	Approximately 350uL of residual cell suspension remains in each tube after the wash procedure
	At the end of the WASH Only procedure, the instrument takes 2 extra minutes to wash the spindle and the tubing; during this time, the cell suspensions cannot be unloaded.

TABLE 2: MANUAL Post Stain method

BD FACSDuet™ Premium Sample Preparation System to Centrifuge	
Process on BD FACSDuet™ Premium Sample Preparation System	Process from specimen dispensing to lyse incubation Carrier was unloaded for post-stain off-board wash
Process for washing using Laboratory Centrifuge	5min Lyse Centrifuge
	Aspiration of lyse supernatant
	Add BD® CellWASH (bulk tank)
	5min Wash Centrifugation (incl. wash disp)
	Aspiration wash supernatant
	Resuspend - add BD® CellWASH
	Preparation Complete - ready to run on BD FACSLyric™ Flow Cytometer

Advantages in having flexibility to do off-board tasks were, but not limited to:

- Use for Lyse-Wash assays on a BD FACSDuet™ Sample Preparation System without washing and centrifugation capabilities.
- Increase throughput with multiple worklists/carriers prepped by one BD FACSDuet™ and/or BD FACSDuet™ Premium Sample Preparation Systems serving multiple BD FACSLyric™ Flow Cytometers.
- Optimize automation of samples requiring off-board steps like stimulation steps and/or incubation at 37 degrees.

Advantages identified for full automation are, but possibly not limited to:

- Standardization, consistency and reproducibility of processing and times.
- Reduction of variability due to manual techniques and staff proficiency levels.
- Risk reduction of manual errors (such as sample exchange or transcription errors).
- Automated traceability of steps, time and users with on-board audit trails.

FIGURE 3: Total Process Time

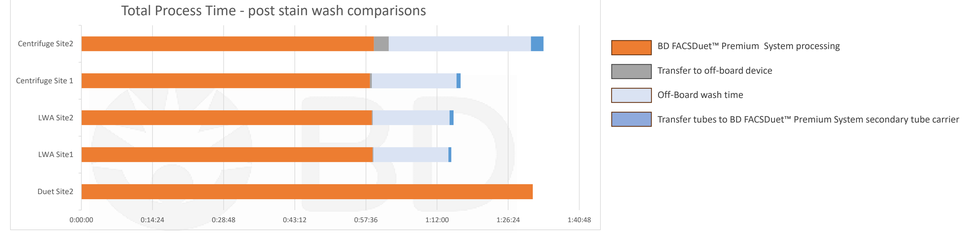


TABLE 4: Total Process Time

Description	BD FACSDuet ™ Premium System Only Site2	BD FACS™ LWA Site1	BD FACS™ LWA Site2	Lab Centrifuge Site 1	Lab Centrifuge Site2
BD FACSDuet™ Premium System processing	1:31:21	0:58:57	0:58:49	0:58:25	0:59:14
Transfer to off-board device		0:00:10	0:00:11	0:00:23	0:03:01
Off-Board wash time		0:15:11	0:15:32	0:17:09	0:28:46
Transfer tubes to BD FACSDuet™ Premium System secondary tube carrier		0:00:35	0:00:49	0:00:49	0:02:32
Total Time	1:31:21	1:14:53	1:15:21	1:16:46	1:33:33
%Difference TPT Site1 to Site2		-0.6%		-21.9%	
Ave TPT	1:31:21	1:15:07		1:25:10	

Site 1: University Hospital Ghent – Belgium
Site 2: ASST degli Spedali Civili Brescia - Italy

Conclusion

- **Predictable workflows were achieved with automation in processing on the BD FACSDuet™ Premium Sample Preparation System or with off-boarding for a post-stain wash using BD FACS™ Lyse Wash Assistant. Processing with traditional centrifugation for post-stain off-board washing showed higher variability between the sites, used more hands-on time, and could introduce errors in processing.**
- **While full process automation did not decrease the total process time compared to the semi-automated processing, it reduced hands-on time and hands-on tasks and the risk for manual errors. Automation provides process standardization across sites, eliminating the variability between users independently of the proficiency level, thus providing process consistency and reproducibility.**
- **Semi-automated processing with off-board post-stain washing with the BD FACS™ LWA, used with the BD FACSDuet™ Sample Preparation System and/or BD FACSDuet™ Premium Sample Preparation System, can provide an automated system with flexibility in protocols that incorporate special requirements, while minimizing the risk of errors and the number of manual interaction.**

The Lean component of this study used timers, paper logging, and video to capture Total Process Tme (TPT), Hands-On Time (HOT), and Error Prone Tasks (EPT) for time and motion.

- Time capture will be from "Press RUN" to "samples ready for acquisition":
- Using calibrated timers, video equipment with the instrumentation to align times across platforms and record time as hh:mm:ss for each step in the process for TPT and HOT. Steps are also assessed whether they are Error Prone.
- Ensure no patient identification is captured in documentation or video equipment.
- Along with video equipment for tracking process, paper documentation is taken immediately during the process that is prepared in advance with the sites SOP's to streamline note taking.
- Lean specialist with background in flow cytometry is crucial in identifying all steps and assessment of error prone steps and deviations from SOP's that may lead to bias in the results.
- The presence of the lean specialist did not interfere with sample preparations and laboratories' Standard Operating Procedures

BD Biosciences provided materials and instruments for this study.