Automated Sample Preparation for laboratories streamlining Lyse-No-Wash and Lyse-Wash methods on a single system using carriers or plates

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

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Introduction

In flow cytometry laboratories, Total Process Time (TPT) using automated sample preparation system physically integrated with a flow cytometer could be different depending on the order of various worklist workflows. Order optimization can increase efficiency and throughput, with the added flexibility of using tube carriers and/or plates.

As these laboratories may perform multiple different test assays, Lyse-Wash (LW) and Lyse-No-Wash (LNW), the ability to provide assay flexibility and to automate as many tests as possible on a single system is important for laboratory efficiency. In addition, advanced automation with the BD FACSDuet™ Premium Sample Preparation System physically integrated with the BD FACSLyric™ Flow Cytometer can reduce error prone steps, increase traceability, and increases walk-away efficiency.

Quantifying TPT for various workflow combinations can aide in identifying the most efficient use of the system to streamline workflow. With human resource strained environments, this can increase throughput for a given instrument and assist in identifying the appropriate complement of instruments for an institution.

Method

Two sites with a BD FACSDuet™ Premium Sample Preparation System physically and data integrated with a BD FACSLyric™ Flow Cytometer explored different workflows. Five (5) different worklists were run in combinations of three (3) and evaluated across four (4) different conditions to assess the overall workflow behavior across various assay types (LW & LNW), carrier types (40 secondary tubes rack & 96-well plate), and centrifuge methods. Time was measured from specimen loading onto the BD FACSDuet™ Premium Sample Preparation System to the completion of acquisition in the BD FACSLyric™ Flow Cytometer. These conditions were compared to each other for workflow efficiency, Total Process Time and Hands-On Time measurements.

Part A: Three different workflow conditions were assessed using two (2) specimens in three (3) defined worklists with various run orders:

- **Worklist A:** LNW assay (BD Multitest™ 6-Color TBNK reagent) prepared in a tube carrier and acquired in BD FACSuite™ Clinical Application (BD-IVD assay)
- **Worklist B:** LNW assay (BD Multitest™ 6-Color TBNK reagent) prepared in a 96-well plate and acquired in BD FACSuite™ Application (non-IVD assay)
- **Worklist C:** LW assay (dried reagent) specimen with off-board pre-wash, to mimic pre-loading cell concentration optimization, with assay prepared in tube carrier and acquired in BD FACSuite™ software (non-IVD assay)

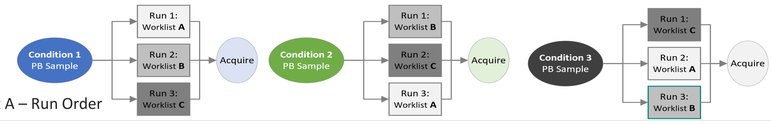

FIGURE 1: Part A – Run Order

Part B: Two (2) different conditions were assessed for processing of 96 samples in different carrier types:

1. three (3) carriers with 32 secondary tubes each were processed concurrently (totaling 96 tubes). Each secondary tube was prepared from one specimen stained with BD Multitest™ 6-Color TBNK reagent (LNW assay) and acquired with BD FACSuite™ Clinical Application (BD-IVD assay)**.**

2. one (1) 96-well plate was processed from 12 specimens (8 replicates each) stained with BD Multitest™ 6-Color TBNK reagent and acquired in BD FACSuite™ Application **(**non-IVD assay**).**

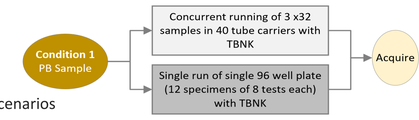

FIGURE 2: Part B Scenarios

Analysis & Results

Part A: Various workflow conditions run concurrently

Of the three (3) workflow conditions run, the most efficient workflow resulted in Condition 3: Worklist C (LW assay with dried reagent) followed by Worklist A (BD-IVD LNW assay with BD Multitest™ 6-Color TBNK reagent), followed by Worklist B (non-IVD LNW assay with BD Multitest™ 6-Color TBNK reagent) (FIGURE 3).

- Condition 3 completed 32.6% faster than Condition 1 where the LW assay was loaded last (TABLE1).
- When running the LW worklist at the end of the three (Condition 1) or as second in the sequence (Condition 2), a time difference of only 5.9% was measured (TABLE1).
- BD FACSLyric™ Flow Cytometer runs worklists in the order of sample preparation completion.

TABLE 1: Total Process Time - complete condition run

Condition	Total Process Time (TPT)	Faster than Condition 1
1	1:58:39	
2	1:51:40	5.9%
3	1:19:55	32.6%

TABLE 2: Total Process Time – Individual run

Condition	Individual BD FACSDuet™ Premium to BD FACSLyric™ run time		
1	0:44:39	0:47:33	1:10:16
2	0:49:48	1:10:57	0:44:48
3	1:16:24	0:43:06	0:49:49

Worklist A (Carrier LNW assay)
Worklist B (Plate LNW assay)
Worklist C (Carrier LW assay)

Observations on Plate vs Carriers

- Plates and carriers can be run interchangeably on the system – FIGURE 3 and TABLE 2
- Regardless where Worklist A (LNW BD-IVD assay) and Worklist B (LNW non-IVD assay in 96 well plate) are run in the consecutive worklists, their BD FACSDuet™ Premium System-BD FACSLyric™ System run time is similar.

FIGURE 3: Concurrent Runs

Observations on assay type:

- The BD FACSDuet™ Premium Sample Preparation System can run up to three (3) worklists and these can be a mixed of both Lyse-Wash and Lyse-No-Wash assays.
- Condition 3 had the LW assay prepared first, allowing for both LNW assays to be prepared simultaneously during the incubation times of the LW one, resulting in the most optimized loading sequence (FIGURE 3).
  - Run time of the LW worklist is slightly longer in Condition 3 in comparison to the other conditions (TABLE 2). Here the LW worklist is prepared first on the BD FACSDuet™ Premium System, yet acquired last. The BD FACSDuet™ Premium Sample Preparation System optimizes the time of all planned worklists, prioritizing some tasks of the LNW worklists (e.g. pipetting of worklists 2 or 3) prior to transferring the LW carrier to the BD FACSLyric™ flow cytometer for acquisition.
  - Operator should be aware of BD FACSuite™ Software requirements for worklist, needing either BD FACSuite™ Clinical or BD FACSuite™ Applications. Condition 3 minimized software Applications changes resulting in increased walkaway time.
- When LW assay was loaded last, as in Condition 1, the worklist did not begin sample preparation until the two previous LNW worklist preparations were completed. LW assays may have a higher number of steps associated with increased movements back and forth to the wash carousel and the on-board centrifuge, ensuring the time indicated for the prep-method is respected, e.g. min. and max. times for reagent and lyse incubations.
- The ability of the BD FACSDuet™ Sample Preparation System and/or the BD FACSDuet™ Premium Sample Preparation System to continually load LNW assays increases throughput and reduces TPT, whether using plates or carriers leading to predictable and reproducible processing times.

Part B: Cross site comparisons: 96 specimens using single 96-well plate or 3 carriers with 32 tubes each

Carriers:

- Three consecutive carriers (96 specimen) throughput at Site 1 had 25.1% lower TPT compared to Site 2. (TABLE 3) When assessing TPT of the process related to the BD FACSDuet™ Premium Sample Preparation System time alone, the carrier inter-site difference was only 1.7%
  - Sample preparation was consistent (hands-on-time [HOT] at 5.9% and 9.5% (Site 1 and 2 respectively as in TABLE 4) with manual intervention at the beginning of the process when loading the BD FACSDuet™ Premium Sample Preparation System
  - TPT difference observed was attributed to sample acquisition.
    - variation in sample types (some samples "timed out" in acquisition due to low cellularity)
    - Sample integrity (age and quality) can contribute to acquisition time differences

Plates:

- Inter-site plate TPT was 2.8% with HOT of 2.3% and 3.4% from Site 1 and 2 respectively.
- Samples run were different with fewer samples "timing out" on acquisition resulting in runs which were more consistent

TABLE 3: Part B – Total process time across sites

96 specimen processing		TPT	TPT %Diff	TPT Duet Only	TPT %Diff Duet Only
Carrier	Site 1	2:52:00	25.1%	2:07:23	1.3%
	Site 2	3:49:46		2:04:23	
Plate	Site 1	3:16:48	-1.7%	1:25:44	4.4%
	Site 2	3:20:15		1:29:29	

TABLE 4: Part B – Hands-On time across sites

96 specimen processing		HOT	HOT%	Average HOT	xdiff
Carrier	Site 1	0:16:25	9.5%	7.7%	2.7
	Site 2	0:13:29	5.9%		
Plate	Site 1	0:06:37	3.4%	2.8%	
	Site 2	0:04:33	2.3%		

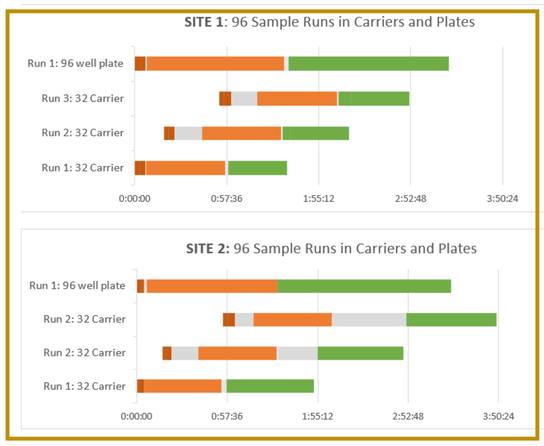

FIGURE 4: Site 1 and Site 2 Throughput for 96 specimens

Automated preparation considerations.

- Many variables need to be taken into consideration when optimizing workflow. To name a few, the minimum and maximum time of sample lysing, whether to batch samples based on arrival time into the lab, and creating preparation methods that use the system in the most efficient manner. Having an optimized workflow can help determine the correct number of instruments a lab will need for their workload.
- Understanding how the BD FACSDuet™ Premium Sample Preparation System prioritizes sample preparation will allow for the most efficient use of the system. Starting a LW worklist first and then batch LNW runs during that prep time increases throughput.
- Limiting the amount of times the flow cytometry software needs to be changed from BD FACSuite™ Clinical to BD FACSuite™ Application allows for increased walkaway efficiency.
- The specimens used for this study were peripheral blood. Other specimen types should be validated for use on the system.

The Lean component of this study used timers, paper logging, and video to capture total process time (TPT), hands-on time (HOT), and error prone tasks (EPT) for time and motion. Time capture will be from "Start of sample prep" to "completion of 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 Total Process Time and Hands-On Time. Steps are also assessed whether they are error prone
- Ensure no patient identification is captures 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 SOPs 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 SOPs that may lead to bias in the results
- Laboratory staff is to perform tasks uninterrupted by the lean specialist to ensure there is no disruption in the times observed or distractions from the SOPs

Conclusion

- The **BD FACSDuet™ Premium Sample Preparation System allows flexibility of processing and loading** configurations of assays, prep methods and workflows, with both carriers and plates.
- Due to **removal of manual interventions, full automation (physical and digital) can be optimized** and customized to achieve predictable process times for each combination of runs and workflows in any given flow cytometry laboratory.
- Sample preparation system **physical integration is advantageous as it increases throughput**, as it standardizes preparation time by removing manual sample transfers to a flow cytometry device.

This research is scientific in nature

BD Biosciences provided materials and instruments for this study.