UNIVERSITY OF TEXAS MEDICAL BRANCH (UTMB) WORKFLOW COMPARISON STUDY WITH THE AQUIOS CL FLOW CYTOMETER

Laboratory Profile

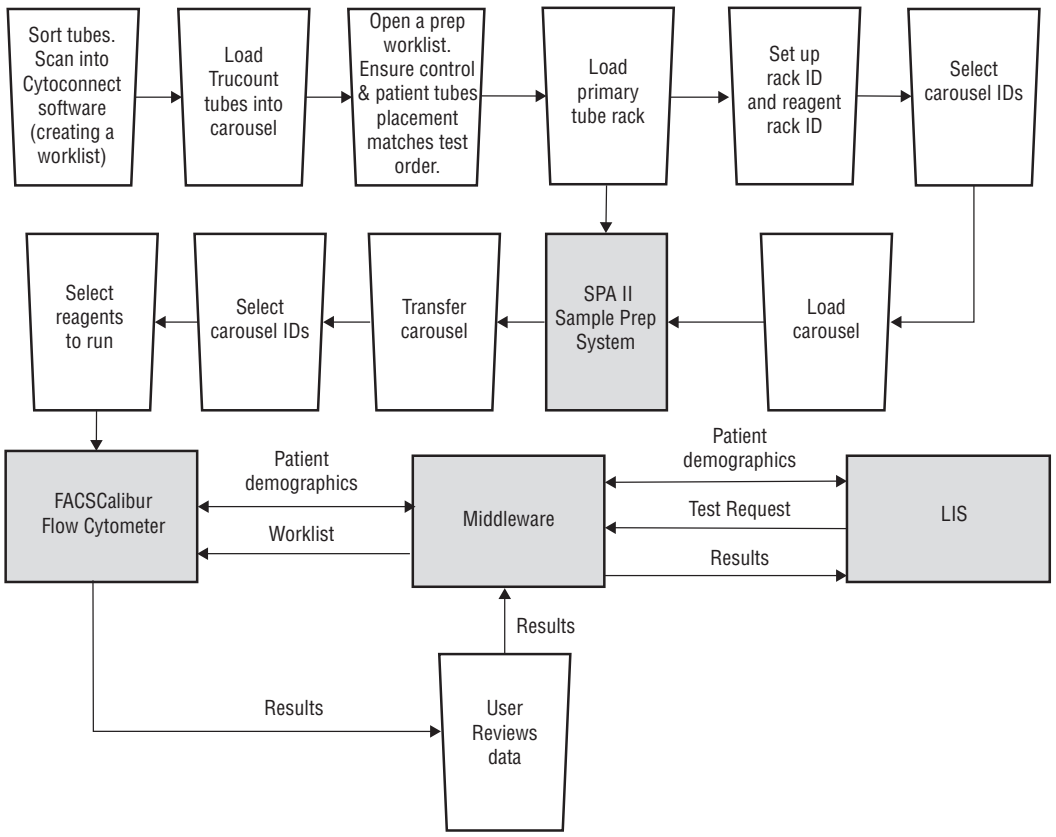
- University of Texas Medical Branch (UTMB), Galveston, Texas, USA
- Flow Cytometry equipment: Becton Dickinson FACSCalibur Flow Cytometer with SPA II system compared to an AQUIOS CL Flow Cytometer

The flow cytometry laboratory at University of Texas is situated on an 84-acre campus with four schools, three institutions for advanced study, a major medical library, a network of hospitals and clinics, an affiliated Shriners Burns Hospital, and several research facilities.

Overview of Traditional Flow Cytometery Workflow

Many traditional flow cytometry systems, such as the current Becton Dickinson FACSCalibur™ with SPA II, require either manual or semi-automated preparation of samples, manual creation of worklists, manual data review, and manual tabulation of numerical data. This workflow can result in longer run times, more hands-on time, and require more experienced operators. See Figure 1 for a Traditional Flow Cytometry Workflow Example.

Figure 1. BD FACSCalibur™/SPA II System Workflow Example



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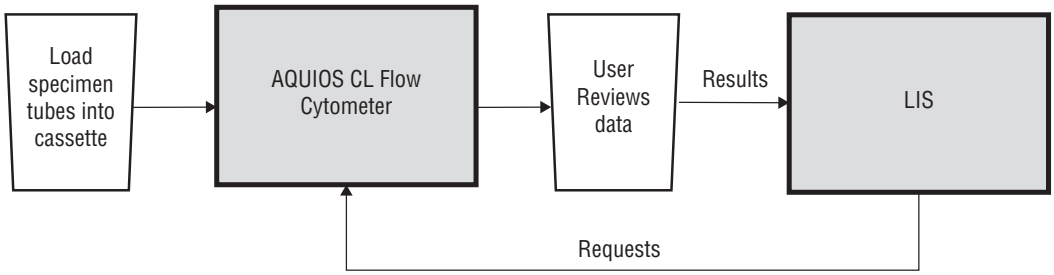
Overview of the AQUIOS CL Flow Cytometer Workflow

The AQUIOS CL system is a fully-automated system that performs all of the preparatory steps leading to a test result. See Figure 2 for the AQUIOS CL Flow Cytometer workflow.

Figure 2. AQUIOS CL Flow Cytometer Workflow



Comparison Protocol

The purpose of this case study is to evaluate the AQUIOS CL Flow Cytometer design and its resultant workflow through direct comparison with the Becton Dickinson FACSCalibur™ and SPA II system. This case study evaluates the following workflow parameters:

- **Total elapsed time from startup to shut down:** The time duration that begins with the first step in starting up the instrument(s) and ends at the last moment of shutting down the instrument(s) when all instrument and software activity has ceased.

- **Time to first result from startup:** For the AQUIOS CL system and alternate systems, the time duration that begins the moment the system is turned on and ends with the first test result for the same sample displayed on the instrument computer screen, for a batch of 10, 25, or 50 samples.

- **Turnaround time:** For alternate systems, the time duration begins with the first sample preparation step and ends with completed test results for a batch of 10, 25, or 50 samples, as demonstrated by the last sample test result displayed on the instrument computer screen. For the AQUIOS CL system, time duration begins with the first cassette placed in the autoloader and ends with the completed test result for the last sample being displayed on the instrument computer screen, for a batch of 10, 25, or 50 samples. The calculation is total time divided by the number of samples. The time does not include QC.

- **Number of procedural steps:** The number of instructions or actions required to accomplish the task. This includes all the distinct steps in the process of generating a test result.

  To avoid individual interpretation of the meaning of a "step," the protocol adheres to the steps as defined by the manufacturer in the applicable Instructions for Use manual. For example, if the Instructions for Use manual states to 'pipette 100 µL of blood,' this is one step. Substeps such as 'insert a pipette tip on the pipette, push down the plunger on the pipette, insert the pipette in the blood sample' are not considered steps unless the manufacturer specifies them as such.

  It is important to note that the AQUIOS CL Flow Cytometer and alternate systems do not employ identical procedural steps. Therefore, the "steps" are categorized by function even if it is not identical system-to-system.

  It should also be noted that, for the AQUIOS CL Flow Cytometer the procedural steps for Tetra-1 and Tetra-2+ tests are identical.

Note: The workflow parameters measured by this case study do not affect safety or effectiveness.

Study Controls

To ensure standardization between both comparison platforms, the following controls were used:

- The same samples were run on both systems.
- Both systems utilized the manufacturer's 4-color, lymphocyte subset panels using BD™ MultiTest™ CD3/CD8/CD45/CD4 for the FACSCalibur™ Flow Cytometer and the AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 for the AQUIOS CL Flow Cytometer.
- BD™ Multi-Check Control and BD™ Multi-Check Low Control were run on the FACSCalibur™ Flow Cytometer and AQUIOS IMMUNO TROL and AQUIOS IMMUNO-TROL Low controls were run on the AQUIOS CL Flow Cytometer for each of the batch numbers studied.
- The AQUIOS CL Flow Cytometer and the FACSCalibur™/SPA II systems were run per the Instructions for Use manuals for each system.
- In all instances, the AQUIOS CL Flow Cytometer utilized the autoloader system.
- In all instances, one individual performed all steps on both systems. The investigator did not multitask between the two systems.
- Control test requests were created prior to initiating the study following the system Instructions for Use manual.

Comparing the FACSCalibur™/SPA II System to the AQUIOS CL Flow Cytometer

Total Elapsed Time from Startup to Shutdown

This test case consisted of 10, 25, and 50 sample scenarios. The AQUIOS CL autoloader system, running AQUIOS Tetra-1 was compared directly to the FACSCalibur™ and SPA II systems, running BD™ MultiTest™ CD3/CD8/CD45/CD4. Note that the 50-test scenario required two batches on the SPA II sample preparation system and three batches on the FACSCalibur™ Flow Cytometer.

Refer to Table 1 for a tabular description of the total elapsed time results. Both the FACSCalibur™ Flow Cytometer and the AQUIOS CL Flow Cytometer demonstrate that larger sample runs (50 samples compared to 10 samples) are more efficient.

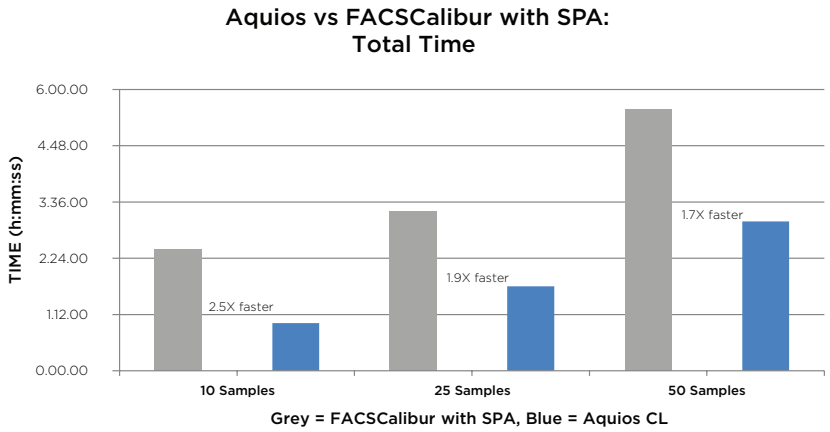
Table 1. Total Elapsed Time

TIME PER STEP DATA	NUMBER OF SAMPLES					
	10		25		50	
	FACSCalibur™	AQUIOS CL	FACSCalibur™	AQUIOS CL	FACSCalibur™	AQUIOS CL
Cytometer Startup and Cleaning	0:12:00	0:10:00	0:12:00	0:09:00	0:12:00	0:12:30
Sample Prep Device Startup and Cleaning	0:06:00	0:00:00	0:04:30	0:00:00	0:04:20	0:00:00
Cytometer Setup, Settings, and Compensation	0:19:00	0:00:00	0:18:00	0:00:00	0:18:00	0:00:00
Sample Prep Device Setup and Processing	1:16:00	0:00:00	1:55:00	0:00:00	3:29:30	0:00:00
Manual Sample Prep	0:00:00	0:00:00	0:00:00	0:00:00	0:00:00	0:00:00
Quality Control	0:04:00	0:02:00	0:04:00	0:01:30	0:04:00	0:01:30
Run Samples	0:19:00	0:35:00	0:33:00	1:23:00	1:06:30	2:40:30
Data Review and Analysis	0:05:00	0:02:00	0:01:00	0:02:30	0:02:00	0:04:30
Clean and Shutdown	0:14:30	0:12:00	0:17:00	0:12:00	0:18:00	0:12:00
TOTAL Elapsed Time	2:35:30	1:01:00	3:24:30	1:48:00	5:34:20	3:11:00

Note: The time format is displayed as (h:mm:ss). For example, 1:01:01 equals one hour, one minute, and one second.

Refer to Figure 3 for a graphic depiction of the total elapsed time results.

Figure 3. Total Elapsed Time



Legend Key: The number after the cytometer name represents the number of samples run.

In the 10 sample scenario, the AQUIOS CL Flow Cytometer is 2.5X faster than the FACSCalibur™ and SPA II system. In the 25 sample scenario, the AQUIOS CL Flow Cytometer is 1.9X faster than the FACSCalibur™ and SPA II system. In the 50 sample scenario, the AQUIOS CL Flow Cytometer is 1.7X faster than the FACSCalibur™ and SPA II system. Assuming 50 samples per workday over the period of one year, the AQUIOS CL Flow Cytometer saves a lab hundreds of hours of valuable time each year. With such a quick turn-around time, the AQUIOS CL Flow Cytometer is capable of more work in less time and has the capacity to do the same work in almost half the time of a traditional Flow Cytometer combination like the FACSCalibur™ and SPA II system. The largest differences are in the areas of cytometer setup and sample preparation device startup, cleaning, setup, and processing. These differences are likely attributed to the fully integrated design of the AQUIOS CL Flow Cytometer that combines the sample preparation device into the flow cytometer.

UTMB reported that the FACSCalibur™ Flow Cytometer 50-sample scenario required two sample preparation runs on the SPA II to complete all of the samples. A similar delay in the workflow can occur on the AQUIOS CL Flow Cytometer once the 96-well plate is full.

Number of Procedural Steps Required

This test case consisted of 10, 25, and 50 sample scenarios. The AQUIOS CL autoloader, running AQUIOS Tetra-1 only, was compared directly to the FACSCalibur™ and SPA II systems running BD™ MultiTest™ CD3/CD8/CD45/CD4. See Table 2 for a breakdown of the results.

The Instructions for Use manuals were used by the investigator to determine the number of steps for each category.

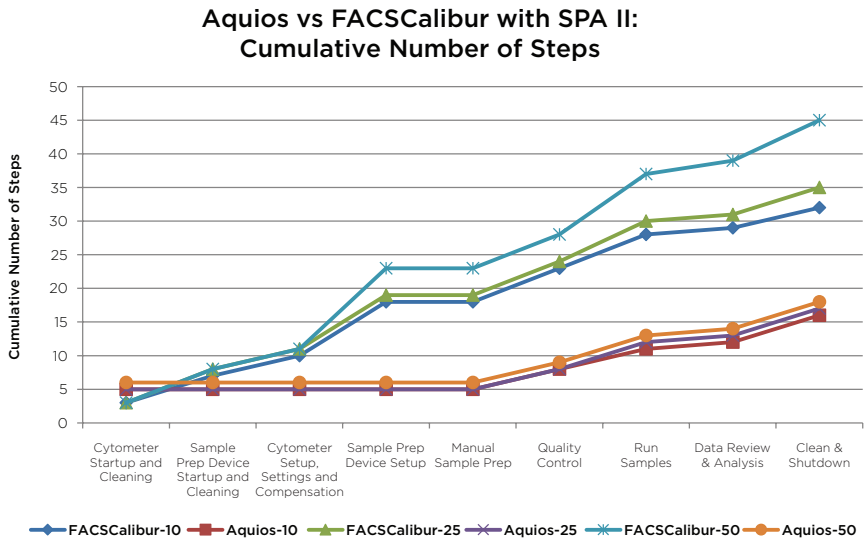
Table 2 and Figure 4 show the number of steps for each specimen

Table 2. Number of Procedural Steps

NUMBER OF STEPS DATA, N	NUMBER OF SAMPLES					
	10		25		50	
	FACSCalibur™	AQUIOS CL	FACSCalibur™	AQUIOS CL	FACSCalibur™	AQUIOS CL
Cytometer Startup and Cleaning	3	5	3	5	3	6
Sample Prep Device Startup and Cleaning	4	0	5	0	5	0
Cytometer Setup, Settings, and Compensation	3	0	3	0	3	0
Sample Prep Device Setup and Processing	8	0	8	0	12	0
Manual Sample Prep	0	0	0	0	0	0
Quality Control	5	3	5	3	5	3
Run Samples	5	3	6	4	9	4
Data Review and Analysis	1	1	1	1	2	1
Clean and Shutdown	3	4	4	4	6	4
Total Steps*	32	16	35	17	45	18

* In the AQUIOS CL Flow Cytometer 25-sample test case, the additional step included the creation of an extra worklist. In the AQUIOS CL Flow Cytometer 50-sample test case the two additional steps included adding new sheath fluid and emptying waste.

Figure 4. Cumulative Steps



Legend Key: The number after the cytometer name represents the number of samples run.

Steps related to sample preparation, quality control, and running samples contributed to a larger total number of steps for the FACSCalibur™ and SPA II system. Regardless of the number of samples in each test case, the AQUIOS CL system consistently reported fewer steps than the FACSCalibur™/SPA II system setup. In addition, the number of steps remained stable across all sample scenarios due to the fully automated design of the AQUIOS CL Flow Cytometer.

Time to First Result, Turnaround Time, and Total Elapsed Time

This test case consisted of 10, 25, and 50 sample scenarios. The AQUIOS CL autoloader, running AQUIOS Tetra-1 only was compared directly to the FACSCalibur™ and SPA II systems running BD™ MultiTest™ CD3/CD8/CD45/CD4.

Three results were measured: time to first result from startup, turn-around time, and total elapsed time.

Refer to Table 3 for the results.

Table 3. Turnaround Time Results

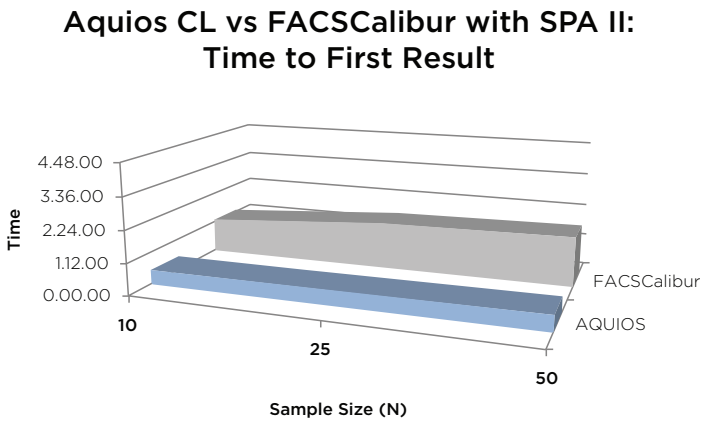
	NUMBER OF SAMPLES					
	10		25		50	
	FACSCalibur™	AQUIOS CL	FACSCalibur™	AQUIOS CL	FACSCalibur™	AQUIOS CL
Time to first result from startup*	1:20:00	0:32:30	1:47:30	0:33:00	1:54:00	0:36:00
Turn-around time**	1:44:00	0:39:00	2:33:00	1:27:00	3:07:00	1:36:30
Total elapsed time	2:35:30	1:01:00	3:24:30	1:48:00	5:34:20	3:11:00

*Sum of cytometer startup & cleaning, quality control, and sample preparation time including time for analysis of the first sample.

**Sum of sample prep device setup, quality control, run samples, and data review and analysis.

Figure 5 depicts time to first result from startup for both the AQUIOS CL system and the FACSCalibur™/SPA II system. The time duration in this instance begins when the system is turned on and ends with the first test result displayed on the workstation. The AQUIOS CL system delivered the first result faster than the FACSCalibur™/SPA II system for all three sample scenarios.

Figure 5. Time to First Result from Startup



Note: The time to first result reported for the 50-sample scenario, unlike the 10-sample and 25-sample scenarios, included the time required to change the sheath fluid. This operation required 3.5 minutes. If the 36.0 minutes were adjusted for this difference, the time to first result for the 50-sample scenario would be 32.5 minutes, which is consistent with the other test cases.

Figure 6 depicts turn-around time for both the AQUIOS CL system and the FACSCalibur™/SPA II system. The time duration in this instance began with the first sample preparation step (FACSCalibur™/SPA II system) or the first cassette being placed on the system (AQUIOS CL system) and ended with the last test result displayed on the workstation. The AQUIOS CL system delivered faster turn-around times in all three sample scenarios. In the 10

sample scenario for turn around time, the AQUIOS CL Flow Cytometer is 2.7X faster than the FACSCalibur™/ SPA II system. In the 25 sample scenario for turn-around time, the AQUIOS CL Flow Cytometer is 1.8X faster than the FACSCalibur™/SPA II system. In the 50 sample scenario for turn-around time, the AQUIOS CL Flow Cytometer is 1.9X faster than the FACSCalibur™/SPA II system.

Figure 6. Turn-Around Time

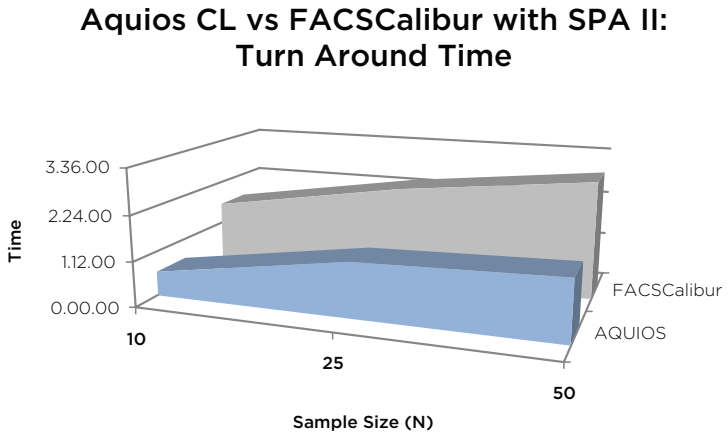
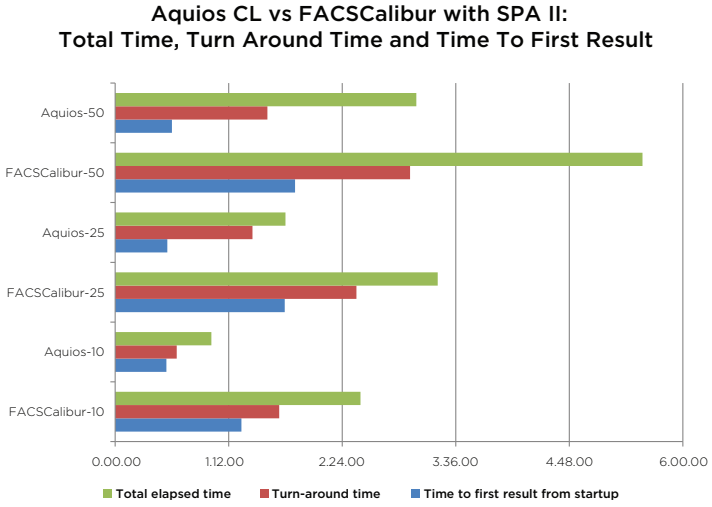


Figure 7 depicts total elapsed time, turn-around time, and time to first result for both the AQUIOS CL system and the FACSCalibur™/SPA II system. In all three instances including total elapsed time, turn-around time, and time to first result from startup, the AQUIOS CL Flow Cytometer consistently delivered faster results in this case study.

Figure 7. Total Elapsed Time, Turn-Around Time, and Time to First Result from Startup



Conclusions

The study indicates that the AQUIOS CL Flow Cytometer performs well compared to a traditional flow cytometry system by providing faster times to first result from startup, faster turnaround times and faster total elapsed times than the comparator, and fewer total steps when processing 10, 25 and 50 samples in the given test cases.

There are two important factors to note regarding this study. The first factor to note is that the time savings exhibited in this study are conservative estimates. A principal advantage of the AQUIOS CL system, freedom from batching, is not measured as a test case. Most current flow cytometry laboratories including FACSCalibur™ system users have

workflows that involve waiting until a critical mass of samples arrive in the lab before starting sample preparations (batching). An AQUIOS CL system user, however, can start running as soon as the first sample arrives. This amounts to improved workflow results in greater time savings than the study indicates. Additionally, the lab can load new samples at any time during the hours of operation with the AQUIOS CL Flow Cytometer. Due to workflow, traditional flow cytometers require samples to be held until the next morning if received past a designated time before closing. The second factor to note is that with many traditional flow cytometers, the operator tends to stay at or near the instrument to monitor progress. If there is not a lot of time in between steps, for example five minutes or ten minutes, the user tends to remain close to the instrument and monitor the data acquisition real-time rather than walk away to accomplish another task. The time that a user is monitoring the data acquisition is not accounted for in this study. The Load & Go nature of the AQUIOS CL system actually accounts for greater time savings and work efficiency than indicated by this study since a user can accomplish more in less time.

Trademarks

The AQUIOS CL is a Class 1 Laser Product

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