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Selection and Validation of Automated Sample Preparation Systems for Clinical Flow Cytometry Laboratories

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INTRODUCTION

Sample preparation is a critical step in flow cytometry, encompassing accurate handling, processing, and labeling of various specimens. With current staffing challenges and the growing complexity of testing, automated preparation systems offer significant advantages. These systems can reduce the workload on laboratory personnel, improve consistency and reproducibility, and minimize human error. Additionally, automated instruments equipped with integrated software can streamline traceability, a task mandated by regulatory bodies that often consumes substantial staff time. Many of these systems are highly adaptable to different assays, allowing laboratories to optimize their resources more effectively.

An overview of the current market offerings of sample preparation systems for flow cytometry can be found in a paper by Al-Attar et al (2024), *Automation in flow cytometry: Guidelines and review of systems* [1]. IVD devices that do both sample preparation and acquisition are out of the scope of this article. Sample evaluation steps before specimen processing, such as visual inspection of sample quality, verification of proper handling, storage, and accurate labeling, are covered in other ICCS modules and CLSI documents.

AIMS

This module aims to guide laboratories in selecting and validating automated sample preparation systems for flow cytometry. Specifically, it simplifies the decision-making process by outlining key considerations, such as the types of assays, specimen suitability, processing capabilities, and

workflow impacts. We herein endeavor to provide thorough guidance on the validation process, including Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ), ensuring that these systems meet manufacturer specifications and laboratory requirements.

EVALUATING AND CHOOSING THE RIGHT SAMPLE PREPARATION EQUIPMENT

It can be challenging to evaluate automated sample preparation devices depending on the needs of the flow cytometry laboratory. Important points include but are not limited to:

- **Type of assays**
    - It is important to evaluate the assays that will be prepared using the sample prep module. Operational analysis may be ideal to determine which assays are the priorities in transitioning to an automated or semi-automated method.
        - IVD assays (e.g. TBNK, CD34)
        - LDT assays
            - Leukemia and lymphoma immunophenotyping
            - Rare event assay (e.g. PNH)
            - Functional assays (e.g. Neutrophil oxidative burst)
            - Other LDT assays not mentioned here (e.g. lymphocyte subset)

- **Specimen types**
    - Careful evaluation should be considered during the assessment of a sample prep system to determine how it can be integrated into the lab's current workflow for different specimen types. Some specimens may have limited volume, low cellularity, or are otherwise unsuitable for automation, while others might need additional processing such as disaggregation and filtering for tissue or marrows. In addition, the lysis of red cells may need to be adjusted for each specimen type or query of neoplasm (e.g. Myeloma or MRD) as discussed in ICCS Quality & Standards Module #1 (Revised 2022): *Lysing Methods and Reagents for Flow Cytometry Immunophenotyping.*

- **Specimen processing**
    - Sample preparation devices may or may not have an integrated centrifuge and/or washing module that can limit the performance of certain protocols. The loading process of various specimen types could also be limited in some instruments and might not allow simultaneous processing or continuous unloading, which can decrease the efficiency of the sample preparation device. There may also be

certain procedural steps that will need to be performed manually (e.g. bulk lysis, pre-washing, or pre-warming) in some options.

- The optimal type of processing protocol must be determined for assay setup [7]
  - Stain → lysis → no wash
  - Stain → lysis → wash
  - Lysis → wash → stain → wash
  - Bulk lysis → wash → stain
  - Wash → stain → lyse → wash
  - Wash → surface stain→ lyse, fix and permeabilize→ cytoplasmic/ nuclear stain → wash

- **Impact on Workflow and Turnaround Time**
  - How many specimens can be loaded in a batch run?
  - How does it handle multiple batch runs? (consider simultaneous batch processing and delay in processing)
  - Can testing be prioritized during processing?
  - Is additional work required to transfer prepared samples to the flow cytometer (e.g., to a carousel or rack, or into different tubes or plates)?

- **Reagent Inventory and Cocktailing Capability**
  - What types of reagents can be loaded (antibodies, lyse and wash buffers, fixatives?
  - Does the system require barcoded manufactured vials?
  - How are reagents maintained on board?
  - Are the antibody reagents refrigerated and protected from light and evaporation?
  - Do the antibody reagents need to be removed daily, or can they stay on the instrument once loaded?
  - How many reagents can be loaded at the same time?
  - What is the minimum reagent volume? (consider handling reagent dead volume)
  - What is the residual volume during and after pipetting? (consider reagent cost and waste)
  - Can the equipment perform cocktailing? (consider productivity improvement and accurate pipetting of antibodies with documentation) [8]
  - Reagent Traceability: Does the system provide traceability for reagent cocktails?

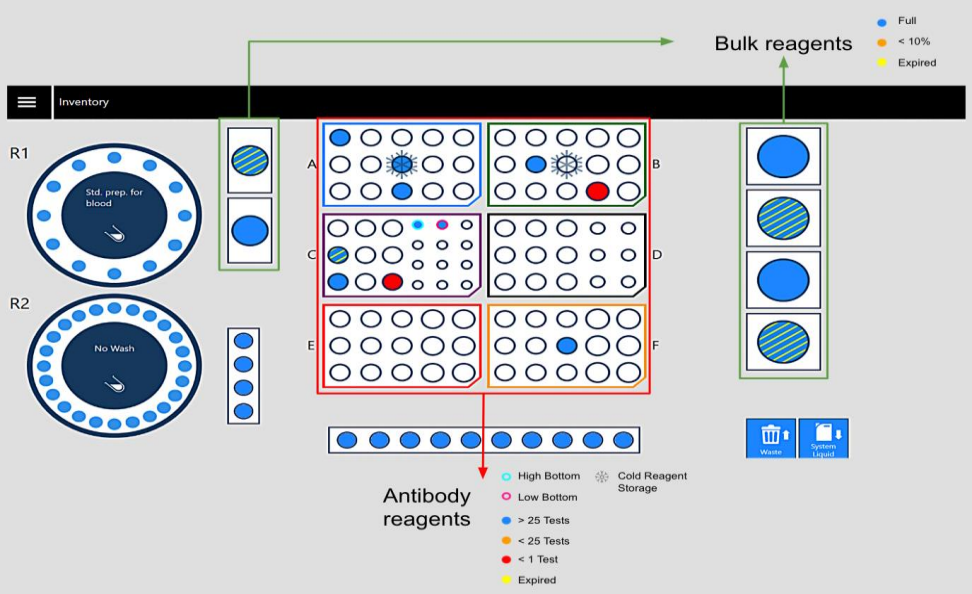

Figure 1. *Example of reagent storage on Sysmex's PS-10, which holds 114 antibodies, 30 of which are refrigerated, protected from light and evaporation, and are left on the instrument once loaded to eliminate the opportunity for mislabeling or switching bottles, and the inventory map on the Sysmex PS-10. One of the considerations in choosing a sample preparation module is how reagents are maintained when loaded.*

- **Laboratory Information System (LIS) Connectivity**
  - Is the equipment capable of LIS connectivity? Retrieving patient and assay information from the laboratory information system helps reduce errors and improves efficiency. It is important to have the IT department involved early on to ensure that this feature can be utilized and to work on a validation system for the interface between the LIS and the sample processing system.

VALIDATION GUIDELINE FOR A SAMPLE PREPARATION MODULE

Guidelines for performing assay validation are available from the CLSI (H42 and H62 Guidelines) and through the ICCS Quality & Standards Committee Modules. While these guidelines are available to use as references, the details of the validation plan are considered to be fit for purpose and are at the discretion of the technical staff and the laboratory's medical director.

A validation plan for automated systems is generally smaller than a full validation plan of a new laboratory-developed test, as it focuses on confirming the performance characteristics established by the device manufacturer. It is recommended that complete qualification reports and other test reports be included as an addendum to the LDT validation report. The validation

phases below provide the summary for each phase and should be supported by appropriate documentation.

Validation for instrumentation and software includes three main phases: Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ). These ensure the correct installation, operation, and performance of the equipment according to the manufacturer and laboratory-specific requirements. Vendors may complete the IQ and/or OQ procedure at installation; however, this might incur an additional cost.

A. Installation Qualification (IQ)

Purpose: IQ is the initial phase of instrument validation that includes the information regarding hardware and software installation, and environmental requirements specified by the manufacturer. IQ is performed by the vendor/manufacturer during installation and is documented accordingly. Documentation may include but is not limited to the following: certificate of compliance (CoC), space requirements, environmental requirements (temperature, humidity, etc.), hazard assessment, hardware requirements, system verification, and electrical requirements. See Tables 2 and 3 for an example of IQ requirements.

Table 1. *Instrument and options documentation: A table detailing the equipment's details, including optional components.*

Identification	Primary Component
Instrument Name	
Manufacturer/Model #	
Serial #	
Manufacturer/Model #	
Serial #	
Clinical Engineering Tag/Asset ID #	
Lab Name for Instrument	

Table 2. *Example of an IQ based on manufacturer requirements. Documentation of the IQ is important to ensure that installation is successfully performed and completed. Evidence can also be included as an attachment since an IQ report can be provided by the vendor.*

Completed by vendor and/or manufacturer:	Pass/ Fail	Performed by	Date
Electric Safety Inspection (ESI): power source requirements, distance from the socket			
Ventilation and Cleaning: space for heat dissipation and cleaning, dust-free as possible, direct sunlight			
Space requirements: sufficient operational space, unit level surface (smooth and level), load bearing capacity (Kg or lbs)			

Temperature and Humidity: operational temperature and fluctuation, relative humidity			
Instrument location: the instrument has its own platform or tethered			
Specified temperature requirements are met for operation.			
Specified humidity requirements are met for operation.			

Additional IQ may also be performed by the institution's clinical engineering department to verify that installation is appropriate and adheres to the facility's requirements. It is also common among institutions to label or tag instruments for regulatory compliance and safety, which should also be documented under IQ. See Table 3 for an example of IQ based on additional institutional requirements.

Table 3. *Example of additional IQ based on laboratory-defined requirements. This documentation verifies that equipment installation is completed.*

Completed by lab personnel and/or engineering department:	Pass/ Fail	Performed by	Date
Has the equipment asset tag been placed?			
Equipment power requirements are assessed			
Temperature/humidity acceptability.			
The effect on other equipment or instruments has been assessed.			
Hazard assessment by the laboratory or clinical engineering			
Information systems and network connections are created			
Laboratory maintenance is created and scheduled			

B. Operational Qualification (OQ)

Purpose: OQ verifies that the software and hardware of the equipment operate according to the vendor's operation qualifications. The OQ can be requested from the vendor/ manufacturer; however, if preferred, the laboratory may choose to perform it independently.

Documentation may include, but is not limited to, the following:

- Calibration details for testing equipment.
- Operational information for instrument hardware and software.
- Functionality checks for level sensors and barcode readers.
- Gravimetric tests to evaluate the accuracy and precision of pipetting steps.
- Interlock functionality checks.
- LIS connectivity verification (if applicable).
- Start-up and shutdown protocols.

- Stress testing the instrument to ensure that it detects operational errors and displays the correct error messages (e.g., operating the instrument without samples or reagents).

Table 4. *Example of OQ based on equipment/device specifications (The following tasks can be rearranged, deleted, or modified depending on the sample preparation system).*

Action (maintenance task/ operational step)	Expected Result	Actual Result	Pass/ Fail	Performed by	Date
Equipment Startup	Completes fluidics initialization				
Equipment Shutdown	Completes daily clean and successfully shuts down				
Priming or Initialization of Fluidics Module	Completes fluidics prime successfully				
Alignment Checks	Completes system check successfully				
System Clean	Completes system clean without errors				
Load Reagent	Successfully reads barcoded reagents or programmed reagents				

Additional support documentation recommended as part of OQ by ICCS Module 17 [6] include:

- Procedures and Forms: a guideline for system operation, performance checks, maintenance, and quality control (QC).
- Training Protocol: instructions covering instrument operation, workflow, quality control, maintenance, validation of the assay protocols, and competency assessment.
- Preventative Maintenance Protocol: routine maintenance plan, software, and system backups that are in alignment with the manufacturer's recommendations.

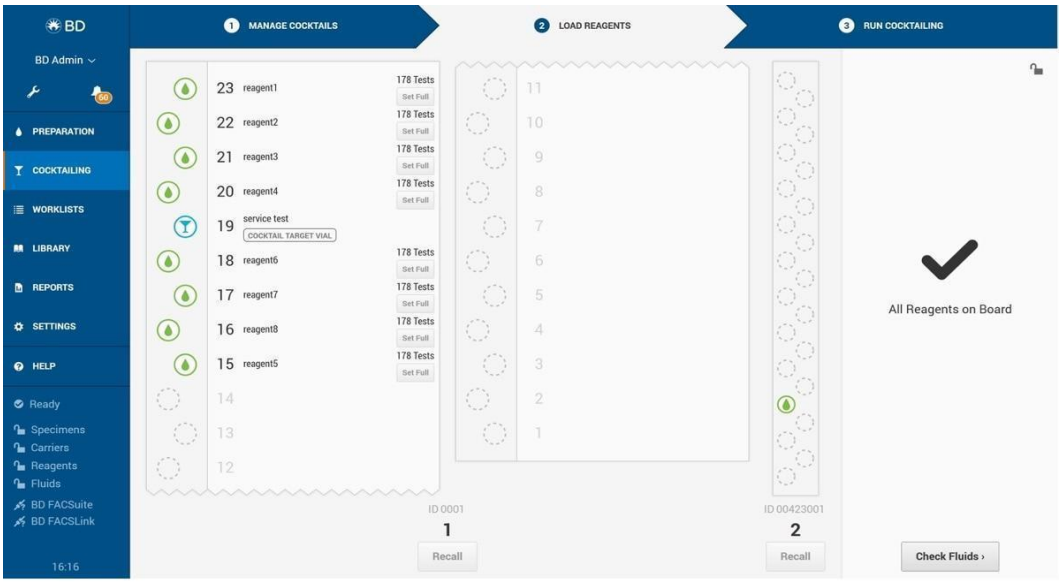

Figure 2. *Example of the screenshot as OQ documentation for testing successful onboarding of reagents using BD FACSDuet Sample Preparation System. Multiple evidence attachments can be included in the validation that verifies the different operational tasks of the equipment.*

C. Performance Qualification (PQ)

Purpose: PQ verifies the laboratory processes and methods that are transferred to the sample preparation device. This qualification is the responsibility of the laboratory. It confirms that the equipment functions according to the manufacturer's specifications and the laboratory's methods and is compliant with all relevant regulatory and accrediting agency requirements. PQ is the final qualification of the equipment and the most extensive phase. This is performed by lab personnel or operators who will primarily use the instrument.

1. Accuracy and Precision (A&P) of Pipetting System

Objective: A&P verifies whether the equipment can properly deliver the specified volumes pipetted by the aspirating and dispensing probes (e.g. specimen probe, reagent probe) integrated into the sample preparation device.

A&P is required at installation but should be verified by the laboratory. The acceptability criteria should be based on the manufacturer's specifications and defined by the laboratory's technical staff and medical director. Table 4 provides an example of how to document and validate the accuracy and precision of the specimen

and reagent probes, typically using deionized (DI) water or PBS. A precision weighing balance, capable of measuring to the thousandth of a gram, is required to measure the weight of the volume before and after pipetting. The scale must be compliant with maintenance and calibration requirements, with proper documentation.

Table 5. *Example of A&P template in verifying a specific volume for the specimen probe (CV= Coefficient of variation). This template can be modified or updated based on the A&P protocol that is defined by the laboratory.*

Accuracy and Precision of Pipetting System			
Type of Probe: Specimen		**Dispensing Volume:** 50 µL	**Accuracy specification** (acceptable weight range)**:** 48.5 – 51.5 µL **Precision specification** (%CV)**:** ≤3%
Tube Number	**Empty (g)**	**Post Pipetting (g)**	**Post Pipetting – Empty * 1000 (µL)**
1			
2			
3			
…	…	…	…
20			
Mean volume (µL)			**Pass/ Fail**
CV (%)			**Pass/ Fail**
Performed by:			**Date:**

2. Carryover Testing

Objective: To assess and quantify any residual contamination in the sample preparation system following routine operation.

Assessing carryover in cellular assays is a critical validation step when integrating a sample preparation system into laboratory workflows. The evaluation should be relevant to the specific assay or panel and tailored to the population of interest (POI). Carryover testing can also assess potential contamination from different system probes (e.g., specimen, reagent, and wash probes) to ensure consistent and reliable sample and reagent delivery without statistically significant residual contamination. Reported carryover ranges could vary between sample preparation systems, so laboratories should establish acceptability criteria aligning with manufacturer specifications and the specimen type and assay context.

This type of carryover testing is typically performed by the vendor during Installation Qualification/Operational Qualification (IQ/OQ), by the laboratory when verification

of the vendor's specifications is required, and at intervals required by regulatory agencies.

For specific carryover testing protocols, laboratories should refer to instrument manufacturers' guidelines and industry best practices, such as CLSI standards H62 and H26-A2. The example of the formula is shown below, where "L" refers to specimens with low target value (low percentage of POI), and "H" refers to a specimen with high target value. Both high and low POI percentage specimens should be tested, with three replicates for each, following sequence: H1, H2, H3, L1, L2, L3 (1,2,3 represent replicates). The carryover is calculated with the formula:

$$Carryover\ \% = (L1 - L3) / (H3 - L3) \times 100$$

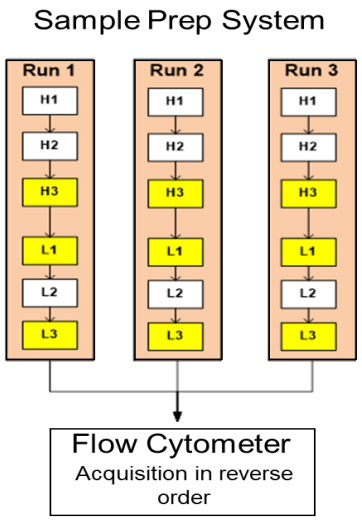

Figure 3. *An example of carryover testing format, adapted from CLSI H62 and H26-A2 guidelines.*

Table 6. *Example of carryover calculation using high ("H") and low ("L") target value specimens. The POI is determined by the technical staff with the laboratory's medical director. An example of high POI can be CD45+ events and low POI can be a blank tube containing PBS, buffer, or water.*

Sample	H1	H2	H3	L1	L2	L3
1	62.65	63.31	62.55	1.87	1.96	1.88
2	63.31	63.44	62.29	1.99	2.18	1.98
3	62.55	63.52	62.15	1.92	2.27	1.88
Average	62.84	63.42	62.33	1.93	2.14	1.91
Carryover (%)	**0.03%**					
Pass/ Fail	**Pass**					

The formula presented above is derived from analytical chemistry, where it is often challenging to create a blank sample due to complex matrices. Using a buffer like PBS as a blank control in flow cytometry is a straightforward approach that streamlines the testing procedure. The following simplified formula can then be applied:

$$Carryover\ \% = \frac{N_{POI\ in\ blank}}{N_{POI\ in\ pos}} \times 100$$

$N_{POI\ in\ blank}$: Number of cells within the POI gate detected in the PBS/buffer

$N_{POI\ in\ pos}$: Number of cells within the POI gate detected in the positive sample

All specimens should be processed according to the standard operating procedure (SOP) for the assay. To minimize potential carryover from the flow cytometer itself, the acquisition is recommended to be performed in reverse order—from low to high POI concentration. The analysis strategy should be applied consistently across all tubes or wells to enumerate cell counts accurately, including gating sequences, gate sizes, and location.

The acceptability criteria should default to the manufacturer's published carryover rate unless the laboratory defines a different threshold. In such cases, the rationale for the alternative acceptability criteria must be justified in the validation documentation and approved by the laboratory medical director.

3. Assay Protocol Verification

Objective: It is recommended to test, verify, and document the new sample preparation method or protocol before starting the sample comparison.

Automated sample preparation systems operate at different speeds compared to manual methods. Some systems employ onboard cell washing methods, such as processing cells in a dedicated onboard washing chamber, directly in the sample tube, or in the well of a multi-well plate. Each step in the workflow, including lysing, staining, and washing, requires careful programming. Parameters such as step duration, and the number of cycles need to be optimized. In addition, some sample preparation systems offer cocktailing functionality beyond standard cell lysing, staining, and washing. Verification of cocktailing protocols is required if this feature is used. An example of a template for an assay protocol that can be modified is shown in Figure 4 and a protocol execution verification is shown in Table 7.

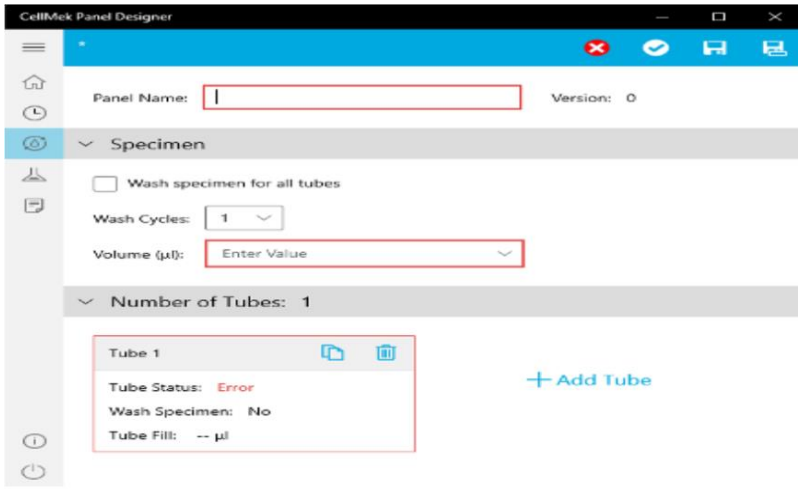

Figure 4. *Example of Beckman Coulter's CellMek SPS panel designer for assay setup protocol that should be verified before clinical use. This documents the specific protocol that is manually created and programmed in the sample preparation module.*

Table 7. *Example of Protocol Execution Verification (The following steps can be rearranged, deleted, or modified depending on the protocol created and the ability of the sample preparation system).*

Assay Protocol Name: _____				
Step	**Description**	**Expected Result**		**Pass/Fail**
1	*Barcode Scan of Loaded Specimen*	*Samples are scanned and shown as a worklist*		
2	*Assign Assay*	*The selected assay was assigned correctly*		
3	*Secondary Tubes in the Sample Carrier*	*The correct number of samples showed in the carrier screen/tab*		
4	*Scanning of Reagents*	*The "Load reagent" tab shows the reagent needed for the assigned assay*		
5.	*Carrier Movement*	*The carrier moved from the carrier drawer to the carrier pipetting position*		
5	*Pipette Liquid*	*Source*	*Specimen Rack*	
		Volume	*__ µL*	
		Destination	*Sample Carrier*	
5	*Pipette Liquid*	*Source*	*Reagent Rack*	
		Volume	*__ µL*	
		Destination	*Sample Carrier*	
6	*Mix and Incubation*	*Carrier is mixed for x seconds and incubated for x seconds/minutes*		
7	*Pipette Liquid*	*Source*	*Bulk Reagent Rack*	
		Volume	*__ µL*	
		Destination	*Sample Carrier*	
8	*Incubation*	*Pipetted specimens are incubated before completion of a task*		
Performed by:				**Date:**

4. Method Comparison

Objective: This confirms that the performance of the new protocol designed in the sample preparation system is comparable to the performance of the current manual protocol.

A comparison between the new automated or semi-automated method and the current or manual method is performed to assess equivalence, quality of results, and stability of the sample prep system. It would be ideal to test various aged samples to verify that mechanical mixing or washing does not compromise the stability of the samples. The details of the validation plan should be fit for purpose and defined with the laboratory's medical director, such as the number of samples tested, and acceptability criteria depending on the laboratory's requirements. Quantitative and qualitative results have different acceptability requirements. It is recommended to acquire a minimum of twenty specimens (n = 20) for comparison with a combination of normal and abnormal specimens.

An example of defined acceptability criteria is shown below (*This should be defined with the laboratory's medical director)*:

- Quantitative test results must be reviewed by the medical director for bias and clinically significant discrepancies in total cell number or cell percentages of selective cell subsets, between the manual preparation and the new sample preparation system. For instance, the loss of plasma cells may affect diagnostic accuracy.
- Qualitative test results should have the same interpretation *(ex. Negative, positive)*.
    - The average qualitative agreement for each parameter across all specimens tested must be greater than or equal to 95% per CLSI EP12 and CLSI H62.
    - Additionally, reviewing cellular phenotype and sensitivity is recommended as automated sample preparation may alter antigen expression signal or background signal due to critical procedures like washing (harsh or insufficient), centrifugation, and decanting.

If the sample preparation system is also used for cocktailing, results from automated versus manual cocktailing can also be evaluated based on the criteria mentioned

above. However, each lab should establish its acceptability criteria based on current criteria for testing lot-to-lot comparison.

Table 8. *Example of method or sample comparison results summary. Tabulated data can also be inserted after the table (The following criteria should be modified depending on the protocols validated and as discussed with the laboratory's medical director).*

Method Comparison Study				
Expected Result	**Actual Result**	**Pass/ Fail**	**Performed by**	**Date**
Cell yield, viability, and selective loss of cell subsets. Percentage difference should be within acceptable limits defined with the laboratory's medical director.				
Sensitivity, LLOD, or LOD is confirmed by using new semi-automated protocol				
Spiking experiment is performed and within acceptable limits. Per CLSI H62, B-cells and plasma cells require a sensitivity of 0.1%, T-cells 1%, and myelomonocytic cells 0.5%.				
Percent difference ____ (*defined with the laboratory's medical director)* across all parameters for the specified test that the assay protocol is performed.				
Correlation between two methods is consistent and bias is acceptable. Appropriate statistical methods are applied (*defined with the laboratory's medical director).*				

5. Precision study

Objective: This ensures consistent and reproducible results from one or across multiple sample preparation systems.

Although the sample preparation module is expected to perform more precise pipetting, it is important to verify precision during validation. For intra-run precision or repeatability, and inter-run precision or reproducibility, a multi-factorial design could be developed to allow multiple factors to be evaluated in a single setting. It is suggested to use three to six samples with a mixture of normal and abnormal specimens. An example of this involving three samples (n = 3) and two sample preparation devices is shown in Figure 5.

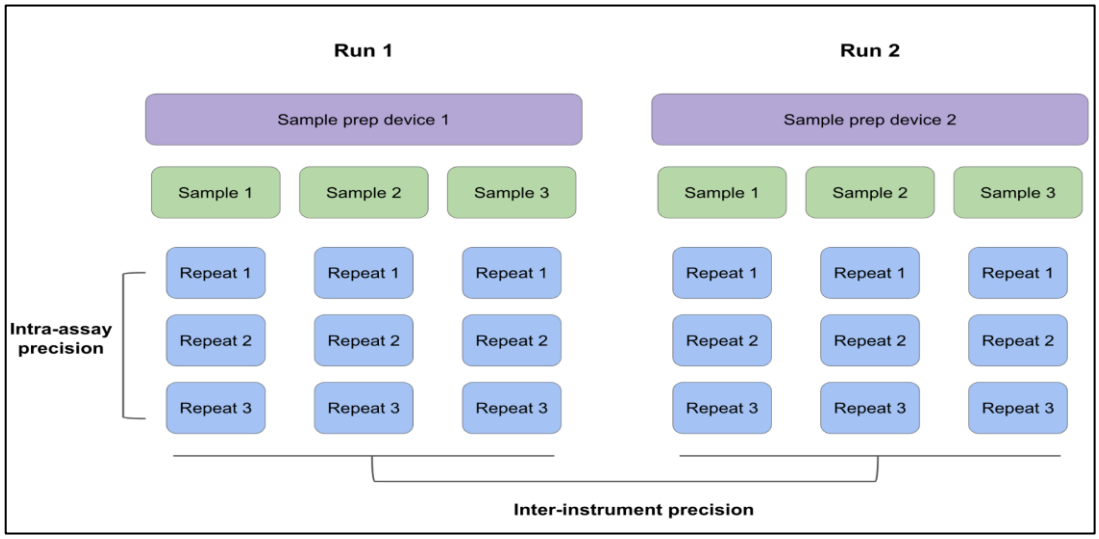

Figure 5. *An example of a multi-factorial design for precision/reproducibility testing, adapted from CLSI H62 guideline.*

Precision studies are performed on semi-quantitative assays that will be prepared (in replicates) using the sample prep module. This allows for the calculation of the coefficient of variation (CV) to determine acceptable precision. A run, or analytical run, is defined as all steps from sample preparation and acquisition. Intra-assay precision measures the variability between the replicates from the same sample processed by the same preparation device. The precision acceptance criteria are suggested to be within 10% to 25% CV. Inter-assay (instrument in this context) precision compares the means and calculates the CV from samples processed with different sample preparation devices (e.g., Run 1 and Run 2). An example of a tabulated result for a precision study that can be used is shown in Table 9. Under certain circumstances, such as for IVD assay verification, repeatability and reproducibility testing must be conducted over several days according to CLSI documents EP5 and H62 Appendix A. A minimum of six samples is required for repeatability testing, and at least three samples are needed for reproducibility testing (These numbers include quality control samples). Examples of single and multiple instrument(s) studies are shown in Table 10 and Table 11.

Table 9. *Example of precision study calculation using a TBNK (T cell, B cell, NK cell) assay protocol for lymphocyte subset analysis. Acceptability criteria should be defined by the technical staff with the laboratory's medical director.*

Precision Study: TBNK Acceptability criteria: CV < 10% across parameters							
Run	**Replicate**	**%CD3**	**%CD4**	**%CD8**	**Abs CD3**	**Abs CD4**	**Abs CD8**
1	1						
	2						
	3						
2	1						
	2						
	3						
Mean							
SD							
CV							
Pass/ Fail							
Performed by:					**Date:**		

Table 10. *Example of single precision studies accounting for day-to-day variability. The single-instrument study comprised seven samples: two quality controls and five patient specimens (C1 and C2: Quality Control samples, such as CD-Chex, Immunotrol, and ClearLLab. S1-S5: Patient specimens)*

Instrument ID	**Day**	**Sample ID**	**Analytical Run #**	**Rep 1**	**Rep 2**	**Rep 3**	**Repeatability (Intra Run)**			**Reproducibility (Inter-Run)**		**Average % CV**
							Mean	**SD**	**% CV**	**Average Mean**	**Average SD**	
Unit 1	**1**	**C1**	**1**									
	2	**C1**	**2**									
	3	**C1**	**3**									
	4	**C1**	**4**									
	5	**C1**	**5**									
	1	**C2**	**1**									
	2	**C2**	**2**									
	3	**C2**	**3**									
	4	**C2**	**4**									
	5	**C2**	**5**									
	1	**S1**	**1**									
	2	**S2**	**1**									
	3	**S3**	**1**									
	4	**S4**	**1**									
	5	**S5**	**1**									
				Minimum % CV						**Mean % CV**		
				Maximum % CV								
Overall Reproducibility				**Acceptance Criteria: % CV ≦ 10%**						**PASS/FAIL**		
				Performed by:							**Date:**	

Table 11. *Example of multiple instrument precision studies accounting for day-to-day variability. The multiple-instrument study included four samples: two quality controls and two patient specimens (C1 and C2: Quality Control samples, such as CD-Chex, Immunotrol, and ClearLLab. S1-S5: Patient specimens).*

Instrument ID	Day	Sample ID	Analytical Run #	Rep 1	Rep 2	Rep 3	Repeatability (Intra Run)			Reproducibility (Inter-Run)		Average % CV
							Mean	SD	% CV	Average Mean	Average SD	
Unit 1	1	C1	1									
	2	C1	2									
Unit 2	1	C1	1									
	2	C1	2									
Unit 1	1	C2	1									
	2	C2	2									
Unit 2	1	C2	1									
	2	C2	2									
Unit 1	1	S1	1									
	2	S2										
	3	S3										
Unit 2	1	S1	2									
	2	S2										
	3	S3										
				Minimum % CV						Mean % CV		
				Maximum % CV								
Overall Reproducibility				Acceptance Criteria: % CV ≦ 10%						PASS/FAIL		
				Performed by:							Date:	

Additional IQ, OQ, and PQ can be performed by the laboratory as deemed appropriate by the technical staff and the laboratory's medical director. The following examples for each qualification in this module are found to be important in validating a new sample preparation system based on the experiences of the authors of this module.

SUMMARY

Sample preparation is a critical step in flow cytometry, involving the handling and labeling of specimens. With rising staffing challenges and testing complexity, automated and semi-automated preparation systems, such as automatic tissue grinders, laminar flow technology, and robotic sample processors, offer significant benefits, including reduced workload, optimized workflow, improved consistency or decreased variability, and minimized human error. This module is intended to guide laboratories in evaluating and selecting automated sample

preparation systems to choose the best fit for their needs, addressing factors such as assay types, specimen suitability, processing capabilities, workflow impacts, reagent management, and LIS connectivity. We have addressed the validation process that follows established guidelines, to verify installation, and operational performance against manufacturer specifications, and more importantly, to ensure the system meets the laboratory's workflow requirements. This module provides examples of validating common sample preparation systems that are commercially available. However, the authors are aware that new systems are being created and may be considered in the future. We hope this guideline provides a framework and serves as a valuable resource for the clinical flow cytometry community.

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