Sponsored and reviewed by ICCS Quality and Standards Committee

Title: Qualification of a 10C 3L Navios Flow Cytometer for Clinical Laboratory Testing

Written by: Amr Rajab, Andrea Illingworth and Teri Oldaker

Date: April 10, 2019

Introduction:

Laboratories introducing immunophenotyping services by flow cytometry are required to meet regulatory and accreditation requirements, verify vendors' claims and demonstrate the acceptance of their diagnostic method. The scope of this module is to cover Instrument Qualification (IQ OQ PQ) of the Beckman Coulter Navios flow cytometer. Verification and Validation scenarios will be described, but will be covered in detail in other modules addressing the assay-specific intent.

The terms qualification, validation and verification have been used interchangeably and loosely.

- The correct term for instruments and reagents validation is qualification.
- For methods or assays the correct term can be either validation or verification and is dependent on the regulatory category of the assay.
    - ✓ Validation refers to establishing performance characteristics of a Laboratory Developed Test.
    - ✓ Verification refers to verification of established specifications of an In vitro Diagnostic (IVD) assay or already validated assay.

Each of the three processes establishes or verifies specifications and quality attributes of instruments, reagents, or methods. Validation can be used a broader term that incorporates instrument and reagent qualification. Instrument and reagent qualification is followed by method validation/verification.

A validation/qualification plan should be developed and approved prior to the launch of the validation/qualification procedure. This plan should describe the required steps, activities, responsibilities, timelines, and acceptance criteria to qualify an instrument for clinical use.

System Description

The Navios is a 10 color flow cytometer which can simultaneously measure forward scatter, side scatter, and up to ten fluorescent parameters using solid-state lasers at 488 nm, 638 nm and 405 nm. Therefore, the instrument can perform multiparameter analyses of individual cells.

Objective

The objective of this Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ) protocol is to provide documented evidence that the Navios

instrument is installed and operates and performs according to the Laboratory and manufacturer specifications and requirements.
Upon completion of the Installation, Operational and Performance Qualifications (IQ/OQ/PQ), the authorization to release the instrument for use must meet the following conditions:

- All acceptance criteria have been met and all specifications have been verified.
- Any critical deviations encountered have been resolved, and applicable corrective actions have been taken and documented.

IQ/OQ Performed by Instrument Manufacturer

A field service engineer (FSE) may complete an IQ/OQ procedure at an additional cost to the laboratory. The tasks performed during this procedure are the same as those done during a standard Navios installation. However, if an IQ/OQ is procured, additional documentation is provided to the laboratory.

A - Installation Qualification (IQ)

Purpose: To establish that the instrument and its components are received as designed and specified, properly installed in the selected environment, and that the environment in which it was installed is suitable for its operation and use.
Installation qualification is divided into two steps: pre-installation and physical installation. During pre-installation, the site is checked for the fulfillment of the manufacturer's recommendations (e.g.: electrical requirements, environmental conditions, vibration level and safety features) in addition to verifying sufficient space for the equipment itself and shelf space for related documentation (e.g.: SOPs, operating manuals, logbooks etc.).

During physical installation, it is verified that the instruments and their components were all received and in working condition (i.e.: undamaged). Additionally, it is confirmed that all fluidics, electrical, and communication connections are established for the system components as per the manufacturer's recommendations and protocol. (Table 1) Specifications that are unique to the Navios EX model are noted in red italics.

TABLE 1 - UNIT INSTALLATION FIELD CHECK

Installation Requirements	Description	Pass/ Fail
Space and Accessibility	**Height**= 60.5 cm (23.8 in.) Additional clearance above for serving and lifting the Data Acquisition card cage above the sensor= 45.7 cm (18 in.) Min. Total Clearance needed= 106.2 cm (41.8 in.)	
	Width= 95.3 cm (37.5 in.) Additional clearance on right for serving= 15.2 cm (6 in.) Additional clearance on left for serving= 15.2 cm (6 in.) Total clearance needed= 125.7cm (49.5 in.)	

	Depth= 70.1 cm (27.6 in.) / *72.6 cm (28.6 in)* Additional clearance behind instrument for sufficient cooling and room for servicing= 3.8 cm (1.5 in.) Total Clearance needed= 73.9 cm (29.1 in.) */ 76.6 cm (30.1 in.)*	
Electrical Input	One dedicated line at 115 Vac, 50/60 HZ at 15 A for Supply Cart Two non-dedicated lines at 115 Vac, 50/60 HZ at 15 A- one for the tower computer and a second, for the monitor, A third non-dedicated line is required for the printer	
Power Consumption	<1500 Watts (N.B. Checked by Beckman Coulter Biomedical engineer)	
Ambient Temperature and Humidity	Room temperature must be between 16c and 32c (60 F and 90 F), and Temp variation should not be more than 5 F per hour. Room humidity must be between 30% and 80%	
Heat Dissipation	Heat dissipation is 720W (2458 Btu/hour). Provide sufficient air conditioning	
Drainage	The waste line from the Cytometer is connected to a 20-L waste container.	
Software functionality	To perform automated system function (i.e. startup & sample feed using automatic mode)	
System alerts	To stress the system to demonstrate that system detects problems and displays appropriate warnings. (e.g. waste full, low Cleanse and Sheath levels, missing sample tube, Carousel Label Read Error, Data Rate Warning…etc)	
Flow Rate	Continuous pressure is applied to the sample tube. The amount of pressure depends on the flow rate you specify: Low approximately 10 uL/min Medium approximately 30 uL/min High approximately 60 uL/min	
Lasers	Solid-state, software controlled, 22 mW */ 55 mW*, blue laser operating at 488 nm Solid-state, software controlled, 25 mW */ 50 mW*, red laser operating at 638 nm. Solid-state, software controlled, 40 mW */ 80 mW*, violet laser operating at 405 nm	
Optical Filters	The filters used in the Navios system are compatible with 10 color phenotyping protocols	
Completed by		**DD/MM/YY**

The following documents are developed as part of the IQ:

1. SOPs and forms for system operation, calibration, maintenance and testing of control materials.
2. A training protocol that provides instruction about operation of the instrument, the workflow in the laboratory, the quality system and document control, instrument maintenance, immunophenotyping testing using the instrument and competency assessment after initial training.
3. A preventative maintenance protocol that is in compliance with the manufacturer's recommendations.

The laboratory should confirm that the Installation Qualification (IQ) is completed and all requirements were met before initiating Operational Qualification (OQ).

B - Operational Qualification (OQ)

Purpose: To verify that the equipment operates according to the vendor's specifications. To test the equipment to establish confidence that it meets all defined user requirements under all anticipated conditions of use as intended by the vendor. (Table 2) Page numbers correspond to version 773232AJ of the Navios IFU (Instructions for Use).

Table 2 - OPERATIONAL FUNCTIONAL VERIFICATION (Navios Flow cytometers)						
		Acceptable				
No.	Description	Yes	No	Initials	Date	Comment
DAILY STARTUP						
	Reagent and Waste levels				DD/MM/YY	See User Manual P. 6-2
	Power computer and cytometer ON				DD/MM/YY	See User Manual P. 6-3
	Alarms associated with results				DD/MM/YY	See User Manual P. 13-1
QUALITY CONTROL						
	Running Flow-Check Pro				DD/MM/YY	See User Manual P. 7-3
	Running Flow-Set Pro				DD/MM/YY	See User Manual P. 7-3
	Instrument Calibration				DD/MM/YY	See User Manual P. 7-3
	Alarms associated with results				DD/MM/YY	See User Manual P. 13-1
RUNNING SAMPLES:						
	Running Sample –MCL Automatic Mode				DD/MM/YY	See User Manual P. 8-2
	Running Sample –MCL Manual Mode				DD/MM/YY	See User Manual P. 8-6
	Running Sample – Single				DD/MM/YY	See User Manual P. 8-11

	Tube Mode					
	Alarms associated with results				DD/MM/YY	See User Manual P. 13-1
DAILY SHUTDOWN						
	Shutdown Instrument				DD/MM/YY	See User Manual P. 6-6
	Power the Computer and Instrument OFF				DD/MM/YY	See User Manual P. 6-7
	Alarms associated with results				DD/MM/YY	See User Manual P. 13-1
MAINTENANCE						
	Cleaning Procedure				DD/MM/YY	See User Manual P. 11-1
	Cleaning Schedule				DD/MM/YY	See User Manual P. 11-1
	Troubleshooting				DD/MM/YY	See User Manual P. 13-1
LABORATORY INFORMATION SYSTEM (LIS):						
	Transmitting Results				DD/MM/YY	

All installation steps are outlined and signed off, and verification data are printed out and signed off by the engineer, as well as documentation of any problems that may have occurred. Also, the training record of the engineer, packing lists, copy of the PO, and declaration of conformity are provided as part of the package given to the laboratory. All sections are also signed off by the laboratory.

Following a Navios installation (whether or not the IQ/OQ is procured), the following mandatory operational tests are completed by the service engineer:

- ✓ Verification of small particle resolution by running 0.5uM beads
- ✓ Running FlowCheck Pro beads 10 times; CVs must be within Beckman Coulter specifications
- ✓ Carryover (must be less than 1%)

We recommend that the laboratory verifies the results of these tests by performing them after the installation is completed, as outlined in this document.

The laboratory should confirm that the Operational Qualification (OQ) is completed and all requirements were met before initiating Performance Qualification (PQ).

C - Performance Qualification (PQ)

Purpose: To verify and document that the Navios Flow cytometer, when operating in its environment, performs it's intended functions in accordance with predetermined documented specifications. It is also to confirm that the instrument functions according to laboratory, regulatory and accrediting agency requirements and consistently with the manufacturer's

claims. PQ represents the final qualification of the instrument and is the most time consuming phase. The PQ is usually performed by the key operator and the lab personnel who will be primary users of the instruments.

a. In the event that a laboratory already has an existing validated IVD or LDT assay and plans to run that assay on a newly qualified instrument, a verification that the assay specifications can be reproduced on the new instrument will serve as the PQ
b. For IVD assays, where the manufacturer has already validated the assay and received clearance from regulatory bodies (such as FDA); a verification that the laboratory can reproduce those specifications in their lab, will serve as the PQ.
c. For new Laboratory Developed Tests (LDTs), the initial method validation serves as a comprehensive PQ. This will be covered in detail in a subsequent Q & S Module.

The following experiments should be included in the PQ:

1. Instrument's Precision and stability study
   Precision studies are done using Flow Check Pro fluorospheres, ran ten times consecutively using fixed PMT voltage settings. The average mean channel for each PMT, the minimum and maximum allowed Mean Channels, and the average HP-CV were calculated. The PMT maximum allowable mean channel drift was < 5% and the average HP-CV for each PMT was within 2SD.

2. Instrument Sensitivity and Linearity
   The sensitivity and linearity for each PMT channel is assessed using a mixture of fluorophores (8 peaks Rainbow beads, Spherotech P/N-998861) with different fluorescence intensities.

3. Small particle sensitivity, debris optimization, and resolution of cell populations
   The small particle sensitivity is tested by the Beckman Coulter field service engineer by running 0.5uM beads. However, the laboratory (in collaboration with a Beckman Coulter Applications Scientist, if available) should confirm this by running a patient sample. If the laboratory will be running T-cell subset analysis (CD3, CD4 & CD8) testing using CYTO-STAT tetraCHROME CD45-FITC/CD4-RD1/ CD8-ECD/CD3-PC5 kit, this will provide the ideal test sample. ImmunoTrol and patients' PB samples should be acquired, as prescribed in the kit IFU. The debris field in the CD45 vs SSC plot, and the resolution between lymphocytes and monocytes, should appear similar to the images found in the IFU. Components of the blue laser light path can be fine-tuned by a Beckman Coulter field service engineer to optimize the appearance of this plot, if necessary.

4. Assessment of Carryover

Carryover is assessed by running Flow Check Pro fluorospheres for 60 seconds. Two tubes containing clean ISO Flow are then run for 60 seconds. Carryover for tubes 2 and 3 should be less than 1%.

5. The PQ part of Instrument Qualification can include data from IVD assay verification or LDT assay validation.

Once the instrument has been qualified, there are different types of method validation or verification scenarios and actions required by the laboratory:

1. New instrument, existing IVD assay
    a. New instrument IQ/OQ
    b. Verify that IVD assay is equivalent to prior instrument results
2. New instrument, existing LDT assay
    a. New instrument IQ/OQ
    b. Verify that LDT assay is equivalent to prior instrument results
3. Existing instrument, new IVD assay
    a. Verify IVD assay results are comparable to the manufacturer specifications
4. Existing instrument, new LDT assay
    a. Develop and optimize new assay
    b. Validate optimized assay on the qualified equipment
5. Existing instrument, modified IVD assay
    a. Modification of IVD assay will reclassify it as LDT (see below #6)
6. Existing instrument, modified LDT assay
    a. Develop and optimize new assay
    b. Validate optimized assay on qualified equipment
    c. Validation will depend on extent of change/modification
7. New instrument, new IVD assay
    a. New instrument IQ/OQ
    b. Verify IVD assay results comparable to manufacturer specifications
8. New instrument, new LDT assay
    a. New instrument IQ/OQ
    b. Develop and optimize new assay

These different validation/verification scenarios will be covered in detail in subsequent Q & S Modules.

Definitions:

1. Qualification: The establishment of confidence that equipment, supplies, and reagents function consistently within established limits. To strengthen control of processes by establishing minimal acceptance criteria and performance for equipment/materials used in this processes
2. Validation: Confirmation by examination and provision of objective evidence that particular requirements (intended use) can consistently be fulfilled. The process of providing documented evidence that the method does what it is intended to do. Validation demonstrates that you are running the right assay.
3. Verification: These are more limited "validations" that are performed for methods, where there is already existing assay and the verification proves that given lab is capable of performing that particular test reliably and precisely.
4. Laboratory Developed Test (LDT): The individual laboratory pathway where a lab develops, optimizes and establishes performance specifications (validates) for an assay. Validation data is not currently submitted for approval; however, the lab can perform this assay on patient samples.
5. In vitro diagnostic test (IVD): The commercial pathway where a company develops, optimizes and establishes specifications (validates) for an assay. The validation is submitted and cleared for approval by a regulatory body (FDA or CE Mark). This allows the company to sell the assay as a system (reagents, instruments, procedures) to a commercial laboratory for patient testing.
6. Assay Modification: A change to an existing assay procedure. Assay modifications can be very minor to very major requiring different levels of revalidation. Modification of IVD assays will change the regulatory category of the assay from IVD to LDT requiring a more rigorous validation.
7. Quality Control: A program that verifies that your established analytic specifications (from validation) continue to be achieved over time. A set of procedures performed by the laboratory staff for the continuous and immediate monitoring of laboratory work in order to decide whether the results are reliable enough to be released. Each assay may require assay-specific quality control measures based on its intended use to demonstrate that the assay is performing according to expectations stated in the assay validation.

Summary

Instruments, reagents and assays inevitably will be upgraded and improved over time in the lab. Regulated laboratories are required to qualify instruments and reagents and validate or verify assays/methods. This module describes the IQ/OQ procedures in detail with example documentation for the Beckman Coulter Navios flow cytometer. The PQ procedure will depend on the assay (whether new or established) as well as the regulatory category of the assay (IVD vs LDT). In certain scenarios where the assay is unchanged with the acquisition of a new instrument, a verification to demonstrate equivalence between the assay run on the new and existing instrument is all that is needed for the PQ. In the event that the assay is significantly modified or the assay is a newly developed LDT assay, a full LDT method validation is required. These scenarios will be covered in in more detail in subsequent modules.

Special acknowledgement: We would like to thank Salima Janmohamed-Anastasakis for her expert advice and contribution to this module.

References

1. CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance - Second Edition – Approved Guideline. Wayne, PA:National Committee for Clinical Laboratory Standards, 2008.
2. CLSI Document H43-A2, Volume 27, No.11. Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline--Second Edition, April 2007.
3. CLSI. A Framework for using CLSI documents to evaluate clinical laboratory measurement procedures. 2d ed. CLSI report EP19. Wayne, PA: CLSI, 2015.
4. CLSI. Laboratory Instrument Implementation, Verification, and Maintenance; Approved Guideline. CLSI GP31-A. Wayne, PA:CLSI; 2009
5. http://blog.fisherbioservices.com/validation-or-qualification-whats-the-difference
6. Navios Flow Cytometer Instructions For Use, Version 773232AJ, June 2018
7. ICCS Quality & Standards Module "Instrument optimization - Adjusting PMT voltages and compensation" https://www.cytometry.org/web/modules/module2.pdf
8. ICCS Quality & Standards Module "Compensation Tips for Beckman Coulter 10-Color Navios Platform" https://www.cytometry.org/web/modules/Module%208.pdf
9. Green, C., Brown, L., Stewart, J., Litwin, V., and McClosky. T. Recommendations for the Validation of Flow Cytometric Testing During Drug Development: I Instruments. Journal of Immunological Methods, 363:104-119, 2011
10. ISAC CYTOU, August 2018
11. Validation the Key to Translatable Flow Cytometry. Part 3- Instrument Qualification. http://cytou.org/store/seminar/seminar.php?seminar=126524

For any questions on this module or any other suggestions, please email info@cytometry.org

Reviewed and approved by: Virginia Litwin and Ben Hedley