Sponsored and reviewed by ICCS Quality and Standards Committee

Title: **Qualification of a 10/12-Colour 3-Laser FACSLyric Flow Cytometer for Clinical Laboratory Testing**

Written by: Ahmad Al-Attar (UK HealthCare, KY)
Katherine A. Devitt, M.D. (UVM Medical Center, VT)

Date posted: June 18, 2020

Introduction

Laboratories introducing clinical testing 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 the BD FACSLyric flow cytometer qualification steps. Qualification of instrumentation includes three components: Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ). These will be described in detail in this module. Assay verification and validation scenarios will be described as related to PQ, 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. However, qualification actually refers to the components used in an assay. Validation refers to the process and results obtained from the assay using qualified components.

- The correct term for instrument and reagent 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 as a broader term that incorporates instrument and reagent qualification. Instrument and reagent qualification is followed by method validation/verification.

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

System Description

The BD FACSLyric™ flow cytometer is available in 4-, 6-, 8-, 10- and 12-color configurations. For the purpose of this module, we will focus on the 10/12 color configurations, both of which 3 lasers—blue, red and violet capable of simultaneous measurement of multiple cellular characteristics. The instrument

analyzes cells as they move in a fluid stream, passing one at a time through a focused laser beam, resulting in fluorescence and light scatter, which is then collected and measured. The BD FACSLyric system consists of a flow cytometer, a computer workstation, and the BD FACSuite™ IVD and Research (RUO) software for data acquisition and analysis, along with the optional BD Assurity Linc™ software, a remote system management software that connects the cytometer with BD technical support personnel for troubleshooting. Optional hardware upgrades include the BD FACS™ Universal Loader, a hand-held barcode scanner, and expanded sheath and waste tanks. The cytometer is equipped with 3 solid-state lasers at 405 nm, 488 nm, and 640 nm. Therefore, the instrument can perform multi-parameter 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 Lyric instrument is installed correctly and that it 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.

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 Lyric installation. However, if an IQ/OQ is procured, additional documentation is provided to the laboratory.

A. Installation Qualification (IQ)

Purpose: Installation Qualification is a process used to verify that all key aspects of system installation adhere to appropriate specifications established by the manufacturer, to establish that the instrument and its components are received as designed and specified, installed properly in the selected environment, and that the environment in which it is 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 and related documentation (e.g., SOPs, operating manuals, logbooks etc.).

Instrument and options documentation: A table detailing the cytometer's details, including optional components is prepared, as in Table 1.

Table 1: Instrument and Options

Instrument			
Model			
Catalog no.			
Cytometer serial no.			
Cytometer manufacturing date			
Universal Loader serial no.			
Universal Loader manufacturing date			
UPS or power conditioner serial no.			
BD FACSuite workstation serial no.			
Data entered by:		***Date:***	

During physical installation, it is verified that the instrument and its components are all received undamaged and in working condition. 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 2).

Table 2: Installation field checks for the BD FACSLyric flow cytometer.

Installation Requirements	Description	Pass/Fail	Initials	Date
Space and Accessibility	Dimensions (W x D x H) Cytometer: 24.9 x 22.8 x 22.8 in. With standard tanks: 33.5 x 22.8 x 22.8 in. With standard tanks & loader: 42.2 x 22.8 x 22.8 in.			
Clearance	Left door x back access x open top 9.8 x 6.0 x 22.8 in			
Total space for cytometer and workstation with clearance	54.8 x 28.8 x 45.6 in			
Total space for cytometer, standard tanks, and workstation with clearance	63.4 x 28.8 x 45.6 in			
Total space for cytometer, standard tanks, workstation, and Loader with clearance	72.0 x 28.8 x 45.6 in			
Power specifications	Voltage: 100-240 ±10% VAC Frequency: 50-60 ±10% Hz Current: 2 A; Power: 150 W			
Operational heat dissipation	≤488 BTU/hour at ambient temperature			
Humidity	15% to 85% relative humidity (noncondensing)			
Operating temperature	15°C (59°F) to 30°C (86°F) Maximum of ±2.5°C/day fluctuation recommended			
Drainage	The waste line from the Cytometer is connected to a waste container			
Software functionality	Performs automated system function (i.e. startup, QC, & sample feed using loader)			

Installation Requirements	Description	Pass/Fail	Initials	Date
System alerts	To stress the system to demonstrate that it detects problems and displays appropriate warnings. (e.g. waste full, low Sheath levels, Loader Size Read Error...etc.)			
Flow Rate	Low: 12 µL/min Medium: 60 µL/min High: 120 µL/min *High sensitivity: 50 µL/min*			
Carryover	<0.10% with default SIT flush <0.05% with 3 or more SIT flushes			
Lasers	Blue laser: 488 nm, 20 mw Red laser: 640 nm, 40 mw Violet laser: 405 nm, 40 mw			
SSC and FSC resolution	Enables separation of 0.2µm beads from noise			
Optical Filters	The filters used in the Lyric system are compatible with 10/12 color phenotyping protocols			

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

Corrective Actions

Corrective actions can be taken by the BD Field Service Engineer for the purpose of correcting failures. These corrective actions must be documented in an Exception Report.

B. Operational Qualification (OQ)

Purpose: The Operational Qualification (OQ) protocol provides documented evidence that all key aspects of functional parameters are operational and adhere to BD specifications and approved design criteria. Additionally, it verifies that any documented recommendations made by BD Biosciences have been applied, and that the environmental requirements are compatible with the operation of the BD FACSLyric system.

To verify that the equipment operates according to the vendor's specifications, and 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, a series of items are tested and checked (Table 3). Details of performing these operations are available in the FACSLyric Reference System document (an HTML resource accessible from within FACSuite software; the "Reference" button on the main menu in both the RUO and Clinical versions).

Table 3: Operational Qualification checklists for the FACSLyric flow cytometer. Details on each of the items listed are available in FACSuite Reference System accessible from within FACSuite software.

Parameter	Specification	Pass /Fail	Initials	Date
Drain and Fill Flow Cell	System drains and fills the flow cell with sheath fluid when selected in the software.			
Purge Sheath Filter	System purges the sheath filter of bubbles when selected in the software.			
SIT Flush	System flushes the SIT, the SIT LEDs change from green to amber, and the flush and aspirator pumps turn on.			
Startup Inspection Checklist				
Inspect the fluidic components and sensors for damage when the instrument is turned on. Make repairs or replacements when necessary	Check for leaks and ensure that all air has been purged from the fluidic lines. Remove bubbles from the flow cell; bubbles can be an indication of leaks in the sheath line.			
	Inspect all quick-disconnects for damage or leaks. Replace O-rings if necessary			
	No air is trapped in the sheath filter.			
	Sheath plenum and waste level detectors are operational			
Fluidic System Operational Checklist				
Sample Flow Rate Verify the sample flow rates (high, medium, and low) using a BD Trucount tube	Low sample flow rate is 8 µL/min - 16 µL/min			
	Medium sample flow rate is 54 µL/min - 66 µL/min			
	High sample flow rate is 108 µL/min - 132 µL/min			
	Sheath flow rate is 13.3 mL/min–14 mL/min Running for 5 minutes is 66 mL–70 mL			
Sheath Flow Rates				
Use a calibrated oscilloscope to measure laser delay at Normal and High Sensitivity modes	Laser delay calibration between the red and blue lasers for Medium flow rate is 34 µs–36 µs			
	Laser delay calibration between the red and blue lasers for High Sensitivity flow rate is 68 µs–72 µs			
	Visually ensure that all lasers are centered in the fiber opening when viewed through a microscope			
Verify BD FACSuite Software Operation				
Demonstrate the functioning ability of the BD FACSLyric computer work-station and the BD FACSLyric electronics, including BD FACSuite Clinical and BD FACSuite software	Microsoft® Windows® operating system executes successfully with no error messages			
	BD FACSuite software connects to the instrument successfully with no error messages			
	BD FACSuite software successfully saves data to a disk.			
	BD FACSuite software successfully prints data on a supported printer			
Digital PMT Voltage Control				
4-3-3 Configuration Measure the PMT voltage response by monitoring the range of light scatter and fluorescence signal intensity of the CS&T beads.	Blue: FSC, SSC, FITC, PE, PerCP or PerCP-Cy™5.5 and PE-Cy™7 When displaying the output as a histogram, changing the channel voltage moves the mean population of the histogram to a different parameter			
	Red: APC, APC-R700 and APC-H7 or APC-Cy7 When displaying the output as a histogram, changing the			

Parameter	Specification	Pass /Fail	Initials	Date
Parameter labels are identified by the instrument configuration.	channel voltage moves the mean population of the histogram to a different parameter			
	Violet: V450, V500 and BV605 When displaying the output as a histogram, changing the channel voltage moves the mean population of the histogram to a different parameter			
Performance Check				
Perform Characterization Quality Check (CQC)	Instrument completes a CQC run without warnings in both BD FACSuite Clinical and Research software.			
Performance Qualification Check (PQC)	Instrument passes the PQC check in both BD FACSuite Clinical and RUO software.			
Lyse Wash Reference Settings	Create LW/LNW Reference Settings in the Setup & QC menu. Make sure that compensation values are within manufacturer-specified ranges in both BD FACSuite Clinical and RUO software			
Verify the System Shutdown Process				
System Shutdown	Run the Daily Clean mode using 10% bleach to DI water solution in the first tube and then using DI water in the second tube. System is primed with water when the option is selected in the software			

During OQ, all installation steps are outlined and signed off, and verification data are printed out and signed off by the engineer. In addition, any problems that may have occurred are documented. 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.

Following a FACSLyric installation (whether or not the IQ/OQ is procured), the laboratory should perfom testing for carryover. The BD FACSLyric's carryover specs are <0.10% with default (single) SIT flush; and <0.05% with 3 or more SIT flushes.

The following documents are developed as part of the OQ:

1. Procedures and forms for system operation, performance check, maintenance and testing of control materials.
2. A training protocol that provides instructions for operation of the instrument, the workflow in the laboratory, quality control, instrument maintenance, clinical flow assays using the instrument, and competency assessment after initial training.
3. A preventative maintenance protocol that is in compliance with the manufacturer's recommendations.

If the vendor performs the OQ, the laboratory should sign off on all the sections to confirm that it is completed and all requirements were met before initiating Performance Qualification (PQ).

C. Performance Qualification (PQ)

Purpose: Performance Qualification performed by the laboratory is the process used to verify and document that the FACSLyric Flow cytometer, when operating in its environment, performs its intended functions in accordance with predetermined documented specifications. It also confirms that the instrument functions according to laboratory, regulatory and accrediting agency requirements, consistent with the manufacturer's claims as well as user requirements. PQ represents the final qualification of the instrument and is the most time-consuming phase. PQ is usually performed by the key operator and the lab personnel who will be primary users of the instruments. There are three possible scenarios:

- **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 showing that the assay specifications can be reproduced on the new instrument will serve as the PQ.
- **For new IVD assays**, where the manufacturer has already validated the assay and received clearance from regulatory bodies (such as the FDA), a verification that the laboratory can reproduce those specifications in their lab, will serve as the PQ.
- **For new LDTs**, the initial method validation on the FACS Lyric serves as a comprehensive PQ.

The PQ part of Instrument Qualification can include data from IVD assay verification or LDT assay validation. The following experiments should be included in the PQ regardless of the scenario:

1. Instrument Precision and Stability Study.
2. Instrument Sensitivity and Linearity: Sensitivity and linearity for each PMT channel is assessed using Cytometer Setup and Tracking (CS&T) beads check.
3. Small particle sensitivity, debris optimization, and resolution of cell populations: Small particle sensitivity is tested by the BD field service engineer by running 0.2uM beads. The data should support that the system is able to separate these beads from debris. However, the laboratory should confirm this by running a patient sample. If the laboratory will be running lymphocyte subset analysis (T, B and NK cells enumeration) using BD Multitest™ 6-color TBNK reagent (CD45:PerCP-Cy5.5/CD4:PE-Cy7/CD8:APC-Cy7/CD3:FITC/CD19:APC/CD16+56:PE) with BD Trucount™ tubes kit, this will provide the ideal test sample. BD MultiCheck Controls and patients' peripheral blood samples should be acquired, as prescribed in the kit technical data sheet (TDS). The debris region in the CD45 vs SSC plot, and the resolution between lymphocytes and monocytes, should appear similar to the images found in the TDS.
4. Assessment of Carryover: Consult with the BD Support for assessment of carryover. BD does not recommend using CS&T beads for measuring carryover unless three SIT flushes are performed after running the beads. The performing lab may develop their own carryover assessment protocol, the principles of which are outlined in the CLSI H52-A guideline.

Once the instrument has been fully qualified, there are different types of method validation or verification scenarios and actions required by the laboratory. These are instrument-agnostic, and will be discussed in a separate module. Some of the above data from the PQ can be incorporated into the validation of a new LDT.

Acknowledgements: The authors would like to thank Silvia Bunting, MD (Cleveland Clinic – Florida), Bruce Grieg (Vanderbilt University Hospital), and John Andreasen (ARUP Labs) for the fruitful discussions and their assistance with this module.

References:

1. Cytometry B, Vol 84B, No5, Sept 2013: Validation of Cell-based Fluorescence Assays: Practice Guidelines from the ICSH and ICCS
2. ICCS Quality and Standards Committee Module #13: Qualification of a 10C 3L Navios Flow Cytometer for Clinical Laboratory Testing. Amr Rajab, Andrea Illingworth and Teri Oldaker.
3. BD FACSLyric™ System Service, Installation & Operational Qualification documentation. ©Becton, Dickinson and Company, 2016.
4. BD FACSuite Reference Manual, v.1.3. ©Becton, Dickinson and Company, 2018.
5. CLSI H52-A: Fetal Red Cell Counting Procedures – Approved Guideline. Wayne, PA: National Committee for Clinical Laboratory Standards, 2001.

Reviewed and approved by:

1. Teri Oldaker, SCYM^{CM}(ASCP) - *Oldaker Consulting*
2. Abigail Kelliher, SCYM(ASCP)^{CM} - *BD Clinical Science Liaison U.S. Marketing*

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