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Title: Instrument Installation, Operational, and Performance Qualification for BD FACS Canto II

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Introduction

BD FACSCanto II is a flow cytometer intended for the qualitative and quantitative measurement of biological and physical properties of cells and other particles to generate multiparametric results for *in vitro* diagnostic use. FACSCanto II system is majorly comprised of a flow cytometer, a fluidics cart, and computer workstation. Flow cytometer utilizes fluidics, optics, and electronics sub-systems to acquire and analyze cells in suspension. Fluidics cart contains operational fluids – FACSFlow cubitainer, FACSClean solution, FACS shutdown solution, and waste container. Computer workstation runs two software packages - FACSCanto clinical software for automated immunophenotyping and BD FACSDiva software for manual immunophenotyping of Laboratory Developed Tests (LDT). The instrument can simultaneously measure forward scatter, side scatter, and up to eight fluorescent parameters using spatially separated 405 nm solid state, 488 nm solid state, and 633 nm HeNe lasers.

Laboratories testing patient specimens by flow cytometry are required by accreditation agencies to document Installation (IQ), Operational (OQ) and Performance Qualification (PQ) of the instrument before bringing the instrument into use. In this document, we briefly outline the steps involved in IQ, OQ, and PQ processes according to the laboratory and manufacturer specifications for BD FACSCanto II analyzers and their critical components.

A. Installation Qualification (IQ)

Installation Qualification is the first phase of instrument validation that provides documented evidence that all key aspects of hardware and software installation adhere to appropriate specifications established by the manufacturer. IQ procedure also verifies that environmental requirements are compatible with the operation of the FACSCanto II system. Calibrated testing equipment must be used to verify installation specifications.

- ✓ *Space requirement:* Before installation of the instrument, laboratory space must be checked as per BD recommendations. BD FACSCanto II is a benchtop analyzer with dimensions of 64 x 91 x 61cm (HxWxD). For operation, it requires additional space on left, right, and top sides for proper air flow and to access main power button and flow cell access door. Fluidics cart with dimensions of 64 x 79 x 61cm (HxWxD) can be installed under the bench. Next to FACSCanto II, enough space is required for computer workstation with either single or dual monitors with an optional barcode scanner with dimensions of 38 x 15 x 23cm (HxWxD).
- ✓ *Environmental requirements:* Room temperature must be between 16 – 30^{o}C with humidity levels at 20 – 80%, and noise levels below 62 dBA.
- ✓ *Electrical requirements:* In addition to space and environmental requirements, dedicated power supply and circuit protection is required for both FACSCanto II and computer workstation.

For physical installation, all components must be verified for any damage. Installation components include

FACSCanto II cytometer with appropriate laser and PMT options, fluidics cart, computer workstation with appropriate versions of BD FACSDiva and BD FACSCanto clinical software, optional FACS Loader, and optional BD HTS. For all components installed, notes should be taken for their physical integrity and if they power up upon connection. Test parameters meeting the specified criteria will be indicated with "Pass", and parameters not meeting criteria will be indicated with "Fail". If any deviations are observed, the executor will complete an exception report including corrective action taken. Installation qualification results and actual readings are further reviewed by instrument validation team to verify if each result is acceptable. Validation team also ensures the proper documentation of reports and screenshots with positive notation of pass/fail/error messages, and executor's initials and date to meet all regulatory requirements.

B. Operational Qualification (OQ)

OQ protocol provides documented evidence that the FACSCanto II system functions within the specified operating ranges set by the manufacturer. OQ procedure also verifies the environmental requirements are compatible with the operation of FACSCanto II system. Calibrated testing equipment must be used to verify OQ testing. OQ testing parameters include:

- ✓ *Software Operation:* Verify FACSDiva and FACSCanto clinical software executes successfully with no error messages, and connects to the instrument.
- ✓ *Fluidics Startup*: Fill BD FACSFlow sheath fluid, BD FACSClean solution, and BD FACS Shutdown solution in appropriate tanks and make sure there are no leaks, sheath and waste level detectors are operational, and system is primed when the option is selected in the software. Make sure fluidics cart air pressure and auxiliary air pressure are at manufacturer's recommendation.
- ✓ *Sample Flow Rate*: Determine sample flow rate by measuring the volume of fluid consumed in 5 minutes at low, medium, and high speeds and make sure BD recommended flow rate is achieved at each speed.
- ✓ *Laser power:* After 20 minutes of warmup, measure laser power for 488nm, 633nm, and 405nm lasers using a calibrated laser power meter and make sure each laser yields the manufacturer recommended output.
- ✓ *Digital PMT Voltage control:* For each parameter, on a histogram, verify the movement of mean population to a different channel as voltage increased or decreased.
- ✓ *Fluorescence Sensitivity:* Sensitivity measures how well the dim populations are resolved from background. Using BD FACS 7-color setup beads, measure the sensitivity of each fluorescence detector and verify obtained sensitivity is greater than manufacturer specified value.
- ✓ *Optical Precision*: Optical precision is the relative standard deviation of a signal produced by identical cells or particles. Using cytometer alignment beads with predetermined coefficient variation (%CV), measure the %CV for each parameter including FSC and SSC and verify the obtained values are less than manufacturer specified value.
- ✓ *BD FACSLoader:* If BD FACSLoader is installed, verify FACSCanto II identifies correct carousal rack number and appropriate tube numbers in the rack. Also make sure all 40 tubes are lifted and acquired without any error messages.
- ✓ *BD HTS operation:* If BD HTS is installed, verify BD HTS communicates with cytometer and FACSDiva software without error messages.

- ✓ *Bar code reader:* If Bar code reader is installed, scan a specimen barcode ID on sample collection tube and verify specimen number matches with the scanned number.
- ✓ *CS&T Performance check:* Using a specified lot of CS&T beads, run the CS&T baseline followed by CS&T performance and ensure instrument completes these runs without errors.
- ✓ *System alerts:* Make sure system alerts the user with appropriate warnings of fluid levels, DAQ warnings, carousal rack ID and missing tube warnings, and run complete alerts.
- ✓ *Fluidics Shutdown:* Run the fluidics shutdown and ensure the system executes the procedure successfully without errors.

Operational qualification can be performed by vendor (BD), qualified internal staff, or contracted external consultants. In our case, IQ and OQ were performed by BD, and internal validation team further reviewed testing parameters if each result is acceptable. Validation team also ensures the proper documentation of reports and screenshots with positive notation of pass/fail/error messages, and executor's initials and date to meet all regulatory requirements.

C. Performance Qualification (PQ)

PQ protocol provides documented evidence that system performs consistently over the period of time for the intended purpose. This is the most time consuming part of the overall validation. PQ should be performed by qualified technologists in the lab. Basic PQ includes Instrument Performance through integrated QC applications, and monitoring linearity and sensitivity over a period of time. Extended PQ includes Inter-instrument comparison, Inter-laboratory comparison, and longitudinal performance.

- ✓ *Instrument maintenance and performance:* Daily maintenance of the cytometer includes running CS&T maintenance module with appropriate lot of beads on DIVA software and running BD FACS 7-color setup beads on FACSCanto clinical software. Review the cytometer report and ensure the run is passed without any error messages or warnings. CS&T baseline report and daily performance report from BD FACSCanto II provides up to 30 metrics about performance of the cytometer including – Linearity, Voltage difference from previous run (delta PMTV), Electronic noise (SDen), optical background (Br), fluorescence detection efficiency (Qr), and %robust CVs for beads in graphical form. Reports also list the laser power and current. If any of the parameters fail to meet the criteria, save the report, and proceed with appropriate troubleshooting. Complete the daily maintenance log with appropriate printouts and screenshots of errors/warnings. After testing is done, run cleaning cycles with appropriate reagents, and shutdown fluidics and cytometer. Weekly maintenance of the cytometer includes instrument surface cleaning, purging fluidic filters, emptying condensation trap, and completing the maintenance log. Monthly maintenance includes performing long clean of the cytometer, cleaning sensors, changing waster container caps, and reviewing Levey-Jennings reports from CS&T and 7-color setup beads. Preventive maintenance is performed biannually by BD service engineer.
- ✓ *Inter-instrument comparison:* Implementing standard operating procedures (SOP) for instrument setup and assay setup and using standardized acquisition and analysis templates reduces variability from instrument to instrument. Inter-instrument performance is evaluated by running the same sample, in parallel, on multiple instruments followed by verifying %CV of results from instruments are within the

established or acceptable range. Acceptance criteria are established during validation and can be regularly reassessed.

- ✓ *Inter-Laboratory comparison:* Global testing of clinical samples on multiple instruments at various sites requires standardized instruments that perform similarly across investigation sites. Application settings based instrument standardization can be implemented by setting target fluorescence values using hard dyed beads such as CS&T beads, 8 peak rainbow beads or Cyto-Cal or Fluorescent Control (FC) beads. Processes aimed at minimizing data variability across instruments and sites can include establishing acceptable ranges for each analyte with quality control (QC) reagents such as stabilized human blood or lyophilized cells, and running QC reagents along with testing specimen with each run. In order for the run acceptance, the QC results should be within the established ranges. Periodically, specimens are split and ship across testing sites and test results should be statistically comparable. To further reduce variability in assay performance, reagents titration and qualification can be performed at one site and distributed across testing sites. New lots of reagents must be evaluated to ensure comparability. Also, cross-train technologists across sites to harmonize practices.
- ✓ *Longitudinal Performance:* Clinical trials are usually conducted over years. In order to maintain quality performance over the time, establish protocols to monitor median fluorescence intensity, linearity, and sensitivity for each fluorescence parameter using calibrated beads (Ex. CS&T beads (BD), Cyto-Cal™ beads (Thermo), rainbow calibration particles (Spherotech), FC beads (BD)). Run these beads on a regular basis (daily, weekly or monthly according to the preferences of the user), and assess instrument performance longitudinally.

Quality control data from the instrument is monitored and reviewed on daily basis to ensure consistency of instrument performance. Proper documentation of reports and screenshots with positive notation of pass/fail/error messages, and executor's initials and date is maintained.

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