Cytometer Harmonization as Part of IVD-R: A Key Component for Performance of Multicenter Studies

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Sooner rather than later, accreditation under the upcoming EU IVD-R 2017/746 Regulation (IVD-R) will be required by all laboratories in the European Union that use or develop in vitro diagnostic tests, including the so-called laboratory-developed tests (LDT), now renamed *in-hospital in vitro devices* (IH-IVD) [1]. Health institutions that use IH-IVD should comply with the ISO 15189 standard, which is an important basis for compliance with the IVD-R [2]. Accurate use of an IH-IVD to ensure performance and correctness requires the implementation of a quality management system, including a quality control system and good laboratory practices, in line with ISO15189, although such an approach is rare in preclinical laboratories [2]. All work must be undertaken in a setting that promotes and ensures reproducibility, enabling results to be translated into useful applications for which standard operating procedures must be put in place. Even though all IVD techniques must adhere to ISO15189 criteria, flow cytometry, and particularly flow cytometry LDTs, are undoubtedly one of the most difficult to accredit.

Flow cytometers are highly customizable instruments, with difficult-to-standardize settings and fluorescence signals that vary between cytometers [3]. In the case of multicolor panels, complexity increases because of assay development, sample preparation, and complex data processing/analysis [4], which in turn hamper performance of multicenter studies. To comply with section 5 of ISO15189 [5], on technical requirements for in vitro devices, and to evaluate the critical parameters, we performed an assay method transfer from a flow cytometer at our facility to a second instrument. The method applied was the basophil activation test (BAT), a flow cytometry–based multicolor in vitro assay that identifies activated basophils after challenge with an allergen or drug [6-8]. We performed correlation studies and determined the level of agreement between both instruments by comparing multiple parameters to identify any critical ones.

From August to October 2022, BAT was performed following standard operating procedures in anonymized clinical samples from patients with confirmed allergy to amoxicillin, clavulanic acid, cefuroxime, ceftriaxone, dipyrone, or azithromycin. Two basic flow cytometers dedicated to routine testing in our in-hospital laboratory were compared (FACSCalibur model 2012: Comparator Instrument [Eq#1] and FACSCalibur Model 2012: Test Instrument [Eq#2])), totaling 430 measures. Activated basophils were assessed using %CD63, %CD203c^{high}, CD203c mean fluorescence intensity (MFI), the CD203cΔMFI,CD203cMFI ratio, and the stimulation index (SI: % marker of stimulated/% marker of nonstimulated cells) [6] (Supplementary material). A cause-effect–based risk approach (as the starting point of a hazard analysis and critical control points) was applied following International Council for Harmonisation guideline Q9 on quality risk management, since it is considered a general tool to identify hazards and risks and to evaluate the impact of changing instruments in the performance of a test [9]. We searched putative critical factors that could affect cytometer performance with consequences for BAT results, thus compromising validation studies. The factors evaluated as potential hazards included photomultiplier tube voltages, MFI, activation molecule (CD63 or CD203c), parameter to quantify activation (% or SI), basophil count, type of culprit drug, sample storage time, and reagent lot. Correlation studies were performed on the continuous variables and on the binary results (positive or negative). The Pearson test was used to evaluate the linear correlation between instruments, Bland-Altman plots to investigate the level of agreement, and the Cohen kappa coefficient (κ) to assess the clinical significance of concordance [9]. The tolerance limits of agreement between instruments were set at κ>0.81 (near perfect agreement).

Testing of critical factors showed that photomultiplier tube voltages could not be transferred from Eq#1 to Eq#2 without drastically changing the results; therefore, they should be optimized individually on each instrument. Importantly, since correlation was not affected by sample storage, type of drug, or culprit, they were not critical factors. The best way to compare both instruments' performance was by matching the MFI and background signal of the nonstimulated samples

of both instruments. We analyzed the different comparators in each cytometer, namely % and SI, using CD63 or CD203c^{high} and CD203c MFI (total, ratio, and ΔMFI). The linear correlation was highest with %CD63 (r=0.9722, *P*<.0001), followed by %CD203c^{high} (r=0.9643, *P*<.0001), and lowest with ΔCD203cMFI (r=0.9494, *P*<.0001). All comparators showed a small bias between Eq#1 and Eq#2, with most of the values falling within the 95% limit of agreement, except for CD203cMFI and CD203cΔMFI (14.33 and 15.61, respectively), indicating good agreement between the instruments. κ indicated near perfect agreement between instruments based on CD63 (threshold, 2.5%) and %CD203c^{high} (thresholds, 2.5% and 5%). In the final test results (n=36), κ ranged from 0.82 to 0.89, indicating near perfect agreement with SI for both markers, and with the percentage for both markers and thresholds ensuring clinical consistency between cytometers, regardless of the sample storage conditions or the culprit used. Study design, gating strategy, concordance, level of agreement, mean (SD) bias and limits of agreement for each comparison, correlation values, and risk-based approach are addressed in the supplementary material. By this risk-based approach, the best comparators were %CD63 (threshold=2.5%), consistent with data reported elsewhere [10], and %CD203c^{high} (both thresholds), since they yielded a good correlation and analytical and clinical κ above acceptance limits, enabling both instruments to be considered equivalent. In turn, using only 1 comparator (ie, CD203c MFI) can lead to nonrepresentative results, highlighting the pertinence of more than 1 readout for robust and reliable results.

Our study aimed to provide clinical laboratories with a roadmap for performing flow cytometry–based in vitro assays as part of their quality system management plan to comply with the IVD-R accreditation process or engage in multicenter studies for which providing reproducible results between all participants is of the utmost importance. Risk-based identification of critical points to compare instruments will facilitate correlation studies and decrease bias, hands-on time, and resource use. Multicenter studies should perform risk analyses to confirm the critical points to be included in external quality programs for ongoing validation of tests and instrument performance.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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