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CROSS-SITE VALIDATION OF A 15-COLOR SPECTRAL CYTOMETRY PANEL FOR LEUKOCYTE PROFILING

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Cross-Site Validation of a 15-Color Spectral Flow Cytometry Panel for Globally Harmonized Leukocyte Profiling in Human Whole Blood for Translational and Multi-Site Clinical Applications

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ABSTRACT

Understanding the complexities of the immune system in health and disease demands expertise and well-defined methodologies. Polychromatic flow cytometry plays a critical role in the precise identification and characterization of immune cell populations, with recent advancements in spectral flow cytometry significantly enhancing this capability.

Ensuring reliable and reproducible results requires the use of rigorously validated flow cytometry panels, particularly when assays are used in multi-center studies. Rigorous validation includes not only intra-assay precision, inter-analyst reproducibility, and inter-instrument comparability at each site but also comprehensive cross-site validation. The latter is crucial in global research and clinical trials, ensuring consistency in data generation across laboratories with different locations and equipment.

This study focuses on the validation of a standardized 15-color spectral pan-leukocyte panel designed to comprehensively identify and quantify major human leukocyte subsets. Validation efforts encompassed intra-assay precision, inter-analyst variability, inter-instrument comparability, and cross-site consistency.

The results demonstrated exceptional intra-assay, inter-analyst, and inter-instrument precision across all analyzed sites. Coefficients of variation (CVs) remained below 25% for non-rare populations and below 35% for rare subsets, fully meeting the recognized acceptance criteria adopted in the field.

Critically, cross-site validation confirmed the assay's robustness in addressing key challenges of global multicenter clinical studies. The method consistently delivered uniform results on the same samples across all participating sites.

These findings underscore the reliability and reproducibility of the 15-color spectral panel, establishing it as a robust tool for research and clinical applications. Successful cross-site validation ensures its suitability for multi-centric studies, enabling consistent and meaningful data integration across diverse locations.

INTRODUCTION

Flow cytometry is a crucial tool for studying the immune system by enabling the simultaneous analysis of multiple chemical and physical properties of individual cells. Its ability to provide detailed, high-dimensional data has made it indispensable in both basic research and clinical applications.

Over the years, significant advancements have been made in the field of flow cytometry. Spectral flow cytometry has advanced the field by capturing the full emission spectrum of each fluorochrome across all lasers in the system, allowing for the measurement of a larger number of parameters within a single cell. This results in enhanced sensitivity, resolution, and accuracy, enabling more precise characterization of immune cells and their subsets.

These innovations have significantly expanded the capabilities of flow cytometry, offering deeper insights into immune dynamics, enabling a comprehensive characterization of the immune system—essential for evaluating therapeutic responses in clinical trials (1–3).

Given the complexity of high-parameter flow cytometry assays and the critical role of flow cytometry in clinical trials - such as assessing drug efficacy, pharmacodynamics, potency, and toxicity - the quality and reproducibility of data rely heavily on the rigorous validation of analytical methods. Factors including sample quality, assay conditions, inter-analyst variability, and instrument standardization can significantly impact measurement accuracy (4).

Furthermore, the global nature of clinical trials, combined with the need to process samples as quickly as possible to remain within the assay's stability window, necessitates the involvement of multiple laboratories capable of performing the same assay while generating consistent data across sites. Although the same type of instruments and standardized conditions are used between laboratories, it is inevitable that each laboratory operates with different equipment and under varying environmental conditions. Cross-site validation is therefore essential to ensure the assay's performance remains reliable across all testing sites. When done successfully, this facilitates the seamless integration of results from diverse laboratories and enhances the robustness of findings in multicenter studies.

A comprehensive validation of a standardized 15-color spectral pan-leukocyte panel has already been described (5). This panel allows for detailed profiling of human leukocytes, with emphasis on key populations such as granulocytes, hematopoietic stem cells, monocytes, and major lymphocyte subsets—T cells, B cells, NK cells, and NKT cells.

The previously published validation demonstrated the robustness of the 15-color pan-leukocyte panel, showcasing consistent precision across all phases of testing (intra-assay, inter-analyst, and inter-instrument evaluations), stable staining performance, and sample integrity maintained for up to 48 hours post-collection. However, those findings pertained exclusively to Site 0, one of the three designated testing sites.

In this study, we expand on this work by presenting a thorough cross-site validation of the same standardized 15-color spectral pan-leukocyte panel. We report intra-assay, inter-analyst, and inter-instrument precision for the two additional testing sites (Site 1 and Site 2), along with an evaluation of cross-site precision, comparing results from Site 1 and Site 2, with the results obtained at Site 0.

METHOD

Panel Components and Sample staining

The 15-color pan-leukocyte panel (Cytek #R7-40007) was utilized to characterize a comprehensive set of major leukocyte populations in human whole blood, including:

- Neutrophils,
- Eosinophils,
- Basophils,
- Hematopoietic Stem Cells,
- Monocyte subsets (Classical, Intermediate and Non-Classical Monocytes)
- T cell subsets (CD4+ and CD8+ T cells)
- B cells,
- NK cell subsets (early, mature and terminal NK cells)
- NKT cells.

The staining protocol was optimized for whole blood collected in sodium heparin tubes and employed a lyse-no-wash assay. This approach, combined with the use of Trucount™ tubes (BD #340334), enabled precise absolute quantification of each population within the blood sample. Details of the antibody reagents used for the panel are provided in **Table 1**.

Streck CD-Chex Plus normal controls were used as testing samples. These commercially available stabilized biological controls, derived from normal human peripheral blood leukocytes and erythrocytes, are designed to resemble fresh whole blood patient samples. Beneficially, this control sample allows identical samples to be tested at different times and locations, when used within the 3 months stability window. By processing the same sample-lot multiple testing sites, the consistency of the sample was ensured, allowing for the verification of assay precision and reproducibility across locations, despite varying testing environments.

Staining was performed on 100 µL of sample CD-Chex Plus normal control. Following staining, lysis, and fixation, samples were stored at 2-8°C, protected from light, until acquisition. Samples were acquired in tubes, on Cytek Aurora Spectral Flow Cytometers the same day as staining (within 6 hours of staining completion).

Before acquisition, Cytek FSP™ Beads were used for optimization, calibration, and quality control of the instruments. Developed by Cytek Biosciences, these beads feature a range of fluorochromes that span the spectral range used in spectral flow cytometry. In addition to aiding in detector alignment and verifying the instrument's ability to resolve signals across multiple channels, Cytek FSP™ Beads serve as internal quality controls, ensuring that the flow cytometer's performance remains consistent and reliable overtime. Moreover, using the same beads, and same lot, across different sites ensures that results remain comparable, even in the presence of variations in instrument types or settings. This approach helps maintain reliable data consistency over time and across different instruments, experimental conditions.

Along with the samples and Cytek FSP™ Beads, one set of library reference controls was acquired at each location for spectral unmixing. The unstained reference control was prepared using the same CD-Chex Plus normal control used for testing. All reference controls were prepared using Cytek® FSP compensation beads (Cytek #SKU B7-10011), except for the CD16 cFluor® B515 reference control, which was prepared using Streck Controls due to the higher expression of this marker on the cells.

Validation Process

The validation process was conducted in Site 1 and Site 2 as performed in Site 0 (5) and based on the guidelines established in H62, Validation of Assays Performed by Flow Cytometry (6).

The following test scripts were assessed (**Figure 1**).

Intra-Assay Precision: Intra-assay precision was evaluated by performing three replicates of the same sample within a single assay at each site. Coefficients of variation (CVs) were calculated for each reportable parameter to measure consistency.

Inter-Analyst Precision: Inter-analyst precision was assessed by having two different analysts per site conduct the same assay on the same day using the same procedure. Each analyst independently prepared three replicates staining mix preparation and carried out all steps of the method. Sample acquisition was performed using the same instrument. Variation between analysts was measured to confirm the reliability of the method across users.

Inter-Instrument Precision: Inter-instrument precision was determined by analyzing the same samples on two different flow cytometers per site. Six replicates of each sample were stained; three replicates were acquired on the primary instrument and three on the secondary instrument. Variation between instruments was measured to evaluate consistent results across different instruments.

Cross-Site Reproducibility: Cross-site validation was performed by using the same lot of CD Chex Plus normal control samples across all testing locations. Lot A (#NL-4211) was used for Site 0 – Site 1 comparison, and lot B (#OL-4155) was used for Site 0 – Site 2 comparison. Each site processed the samples under the same experimental conditions, ensuring that data were comparable despite potential variations in local instrumentation, environmental factors, or handling procedures. This step was critical to confirm that the assay could deliver consistent and reliable results across different geographic locations, facilitating the integration of data from multiple sites in multicenter studies. Variability between sites was assessed to ensure the robustness of the assay for global clinical trials.

Data analysis

To accurately identify and quantify the populations of interest in each validation assay, a comprehensive hierarchical gating strategy was employed.

An initial broad gating strategy (**Figure 2**) was used to exclude periods of fluid instability, debris, and doublets, and to identify Trucount™ beads and CD45+ leukocytes. After that, starting from CD45+ Leukocytes gate, three sequential gating pathways were derived to focus on specific groups of cell populations.

The granulocyte gating strategy (Figure 3) identified granulocyte subsets (neutrophils, eosinophils, and basophils) and CD66b – CD123– cells, referred to as the "NOT[Basophils]" population. These cells served as the starting point for subsequent gating strategies (Figure 4Figure 4) to identify CD34+ HCS, Monocytes cells subsets (classical, non-classical, and intermediate), and Lymphocytes.

For deeper lymphocyte characterization, cells not fitting the CD3-CD19- gate or CD7-HLA-DR+ populations were considered, leading to the identification of CD19+ B cells, CD4+ and CD8+ T cells, CD56+ NK-like T cells, and NK cell subsets (**Figure 5**).

Readouts and acceptance criteria

The hierarchical gating strategy identified 19 populations. For each population, the frequency of CD45+ white blood cells (% of CD45+ WBC) and absolute counts were calculated. Additionally, for all lymphocyte populations, the percentage of lymphocytes (% of Lymph) was determined.

Reportable parameters for each identified population are listed in **Table 2**, totaling 47 readouts (19 absolute counts, 19 frequencies of CD45+ WBC, and 9 frequencies of Lymphocytes).

Readouts were measured in each validation test script and categorized into readouts associated with rare and non-rare cell subsets as outlined in **Table 3**. Non-rare cell subsets were defined as cell subsets with a frequency of CD45+ WBC >10% and at least 500 events in the gate. Rare cell subsets were defined as cell subsets with a frequency of CD45+ WBC ≤ 10% and at least 100 events in the gate, or with a frequency of CD45+ WBC > 10% but with 100 to 499 events in the gate.

The mean and the standard deviation were calculated among replicates in each readout. For precision and reproducibility assessment, the percent CV (% CV) of each readout was calculated among replicates in each test script and then evaluated against acceptance criteria.

The acceptance criterion for non-rare cell subsets was a mean % CV ≤30%. For rare cell subsets, more lenient acceptance criteria are justified by the fact that small sample sizes are statistically less representative of the overall population; low population frequencies and fewer events naturally lead to increased population variance (6–8). Therefore, an acceptable precision range of 40% CV was adopted. Readouts associated with populations having fewer than 100 events in the gate on average were reported but not included in the assay evaluation due to insufficient cell numbers.

RESULTS

Precision: Intra-assay, inter-analyst, inter-instrument

For all the identified populations, the frequency of CD45+ WBC, the frequency of lymphocyte (in case of lymphocytes subsets), and the absolute count were calculated in each replicate of the intra-assay, inter-analyst, and inter-instrument test scripts at Site 1 (**Figure 6, A-B**) and Site 2 (**Figure 7,A-B**). The % CV across replicates at each testing site was calculated (**Site 1, Figure 6, C-D; Site 2, Figure 7, C-D**) and evaluated against the acceptance criteria to assess the qualification status of each readout.

Out of the total readouts assessed, those associated with the CD34+ HSC and Terminal NK Cells populations could not be evaluated in all test scripts due to insufficient gated events, which numbered fewer than 100 and therefore did not meet the threshold for rare cellular subsets.

All remaining evaluable readouts met the acceptance criteria for intra-assay precision, inter-analyst precision, and inter-instrument precision, with % CVs lower than 25% for all the readouts associated with non-rare subsets and lower than 35% for all the readouts associated with rare subsets (e.g. CCR3+ Eosinophils in Site 1 inter-instrument assay). Notably, more than 75% of the readouts in all the test scripts had a % CV significantly low (less than 5%) at both testing sites (**Figure 8**). Additionally, the % CV for the 5 readouts that could not be assessed due to the lower number of events in the gate was also lower than 25%, thus suggesting that increasing the initial blood volume could allow these data to meet the acceptance criteria as well.

Overall, based on these results, the 15-color pan-leukocyte panel demonstrated validated precision across intra-assay, inter-analyst, and inter-instrument tests at each site.

Coss-Site Reproducibility

For all the identified populations, the % CV was calculated using results from 3 replicates processed at Site 0 and from 3 replicates processed at Site1 or Site 2. Consistent the precision tests, readouts associated with the CD34+ HSC and Terminal NK cell populations could not be evaluated due to insufficient gated events. All remaining evaluable readouts were evaluated against the acceptance criteria to assess the qualification status of each readout. All %CV values met the established acceptance criteria, with the sole exception of the CD45+ cell frequency within the rare CCR3+ eosinophil subset, which exhibited a %CV of 35.3 in the site 0–1

comparison, slightly exceeding the predefined threshold. Notably, more than 75% of the readouts in all the test scripts had a % CV lower than 10% at each testing site (**Figure 9A**).

To further confirm the reproducibility of the assay across sites, we evaluated the Pearson correlation between the mean of the values measured at Site 0 and the mean of values measured at Site 1 and Site 2 (**Figure 9B**).

The Pearson correlation coefficient between 0.9 and 1 in all the comparisons highlighted the strength of the relationship between data generated at different sites. The line of best fit, included in the graph, further confirms this trend.

In conclusion, these results demonstrate that the validation of the 15-color pan-leukocyte assay exhibited not only high precision but also outstanding reproducibility across multiple sites.

CONCLUSION AND DISCUSSION

Our results underscore the robustness of the 15-color pan-leukocyte panel, with consistent precision observed across all validation parameters and testing sites. Precision, or repeatability, is a crucial metric in flow cytometry validation. Assays that lack high precision demand cautious interpretation and may require larger datasets to differentiate measurement variability from biological variability. The minimal variation observed in the intra assay test script, as well in the inter-analyst validation, with over 90% of CVs below 25%, reflects not only the effective and essential implementation of standard operating procedures, but also the effectiveness of a well-executed training program.

Inter-instrument variability is another important source of imprecision, especially in global studies involving multiple testing sites. To ensure reproducibility, instrument qualification, calibration, daily setup, and standardization are critical(9). The integration of globally standardized operating procedures, comprehensive training, and rigorous instrument calibration and standardization enabled the assay to achieve exceptional precision and reproducibility across multiple sites.

Rigorous evaluation during validation guarantees that the assay performance is consistent despite differences in analyst, instrumentation, and environmental conditions enabling reliable integration of data across locations into a unified analysis, thus allowing to obtain robust conclusions from complex global studies, where data reliability and integrity are paramount for taking critical decisions.

Overall, the cross-site validation of the 15-color spectral flow cytometry panel demonstrates its robustness, precision and reproducibility for pan-leukocyte profiling, making this panel an invaluable tool for immune monitoring in complex, global clinical studies.

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CONTACT INFORMATION

For any questions or requests, feel free to contact us at https://kcasbio.com/contact-us/

TABLES

Table 1: 15-color Pan-Leukocyte Panel

Target	Clone	Fluorophore
CD45	HI30	cFluor® V547
CD66b	G10F5	cFluor® R720
CD123	6H6	cFluor® BYG610
CD193 (CCR3)	5E8	cFluor® BYG667
CD34	4H11	cFluor® BYG575
CD14	MEM-15	cFluor® BYG781
CD16	3G8	cFluor® B515
HLA-DR	L243	cFluor® V505
CD7	CD7-6B7	cFluor® R659
CD19	HIB19	cFluor® BYG750
CD20	2H7	cFluor® R685
CD3	SK7	cFluor® R780
CD4	SK3	cFluor® V610
CD8	SK1	cFluor® V450
CD56	LT56	cFluor® BYG710

Table 2: Readouts List

#	Population	Parent Gate	Readouts
1	CD45+ WBC	Single Cells	Absolute Count[1], % of Single Cells
2	Neutrophils	CD66b+ SSC Hi	Absolute Count[1], % of CD45+ WBC
3	CCR3+ Eosinophils[2]	Eosinophils	Absolute Count[1], % of CD45+ WBC
4	CCR3+ Basophils[3]	Basophils	Absolute Count[1], % of CD45+ WBC
5	CD34+ HSC	CD34+	Absolute Count[1], % of CD45+ WBC
6	Non-Classical Monocytes	CD7-HLA-DR+	Absolute Count[1], % of CD45+ WBC
7	Intermediate Monocytes	CD7-HLA-DR+	Absolute Count[1], % of CD45+ WBC
8	Classical Monocytes	CD7-HLA-DR+	Absolute Count[1], % of CD45+ WBC
9	Total Monocytes[4]	CD7-HLA-DR+	Absolute Count[1], % of CD45+ WBC
10	Lymphocytes	NOT-[CD3-CD19-] OR-NOT-[CD7-HLA-DR+]	Absolute Count[1], % of CD45+ WBC
11	CD20+ B Cells[5]	CD19+ B Cells	Absolute Count[1], % of CD45+ WBC, % of Lymph
12	Early NK Cells	CD7+	Absolute Count[1], % of CD45+ WBC, % of Lymph
13	Mature NK Cells	CD7+	Absolute Count[1], % of CD45+ WBC, % of Lymph
14	Terminal NK Cells	CD7+	Absolute Count[1], % of CD45+ WBC, % of Lymph
15	Total NK Cells[6]	CD7+	Absolute Count[1], % of CD45+ WBC, % of Lymph
16	CD3+ T Cells	Lymphocytes	Absolute Count[1], % of CD45+ WBC, % of Lymph
17	CD8+ T Cells	CD56-CD3+	Absolute Count[1], % of CD45+ WBC, % of Lymph
18	CD4+ T Cells	CD56-CD3+	Absolute Count[1], % of CD45+ WBC, % of Lymph
19	NK T Cells	CD3+ T Cells	Absolute Count[1], % of CD45+ WBC, % of Lymph

[1] Absolute count is reported as cells/mL
[2] Referred to as 'Eosinophils' for absolute count readouts
[3] Referred to as 'Basophils' for absolute count readouts
[4] Defined as the sum of the classical, intermediate, and non-classical monocyte subsets
[5] Referred to as 'B Cells' for absolute count readouts
[6] Defined as the sum of the early, mature, and terminal NK cell subsets

Table 3: Rare and Non-rare cell subsets definition criteria

Subsets classification	% of Parent	# Events
Non-rare cell subset	> 10%	>500
Rare cell subset	>10%	100 - 500
	≤ 10%	>100
Exclusion criteria	-	≤ 100

FIGURES

Figure 1

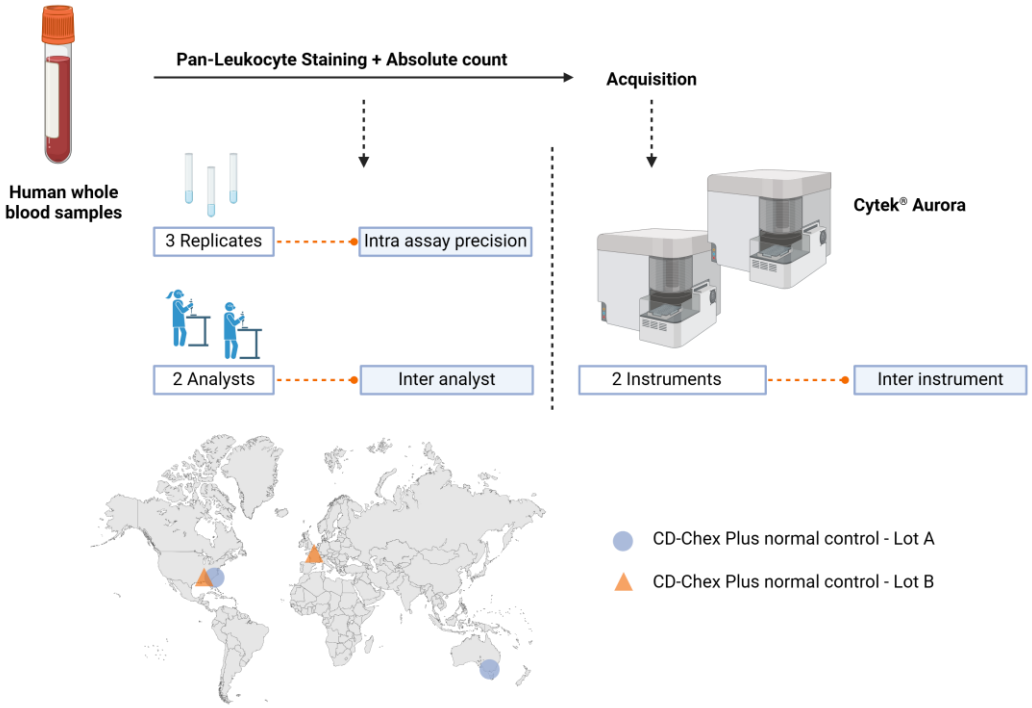

Figure 1: Schematic Overview of Experimental Workflow for the Validation of the 15-Color Pan-Leukocyte Panel. Intra-assay Precision, inter-analyst Precision, inter-instrument Precision, were tested. Streck CD-Chex Plus normal controls were used as testing samples, stained with the Pan-Leukocyte Panel combined with the absolute count quantification. Three replicates from each sample were assessed in each test script. Intra-assay precision was assessed among the three replicate tubes. For the Inter-analyst precision, replicates of the same samples were stained by two different analysts on the same day. For inter-instrument precision, six replicates per sample were stained, then three replicates were acquired on the primary Cytek® Aurora instrument and the other three on the secondary Cytek® Aurora. Cross-site validation was performed by using the same sample-lot across two testing locations. Lot A was used for Site 0 – Site 1 comparison, and lot B was used for Site 0 and Site 2 comparison.

Figure 2

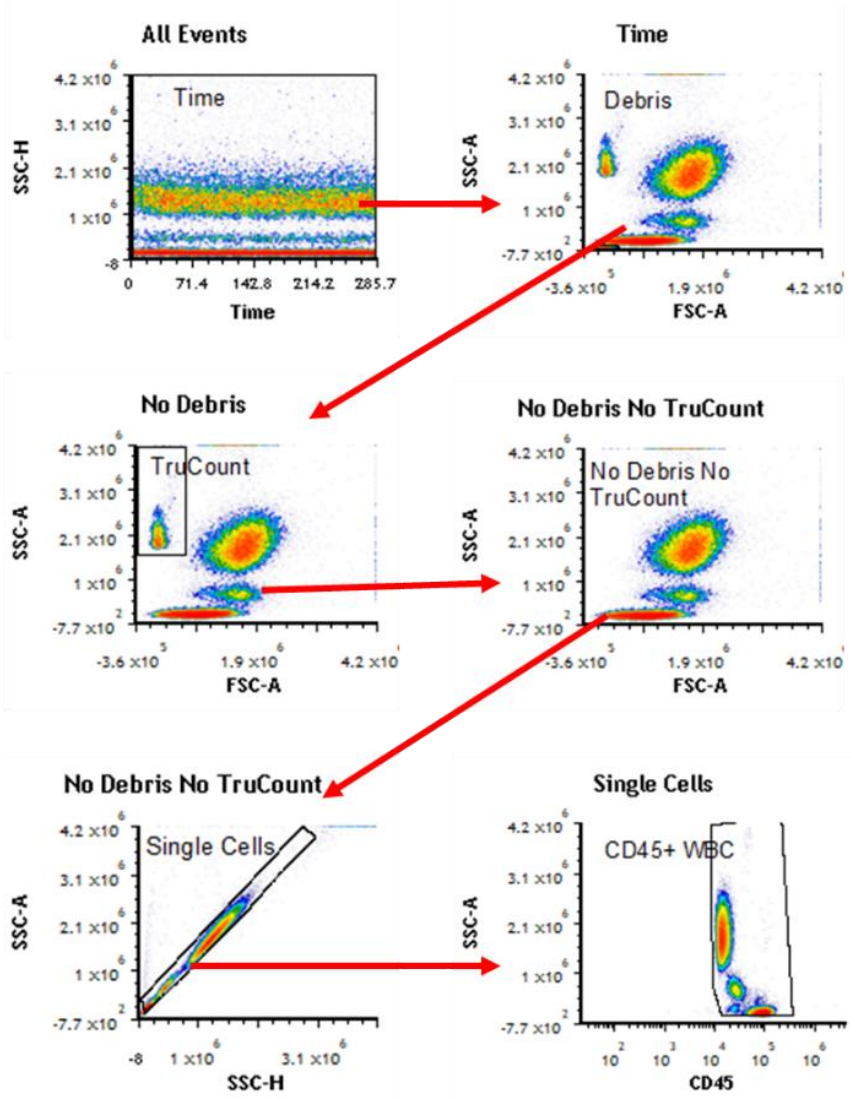

Figure 2: Preliminary Gating Strategy. A QC Time gate (SSC-A versus Time) was used to exclude periods of fluid instability from the analysis. Debris was removed by gating based on FSC-A and SSC-A, selecting events outside this gate (NOT-[Debris]) for further analysis. Trucount™ beads were gated from the debris-negative events, and a NOT gate was applied to define non-debris, non-Trucount™ bead events for subsequent analysis. Singlets were identified based on the linear relationship between FSC-A and FSC-H. Leukocytes (CD45+) were then identified based on CD45 expression.

Figure 3

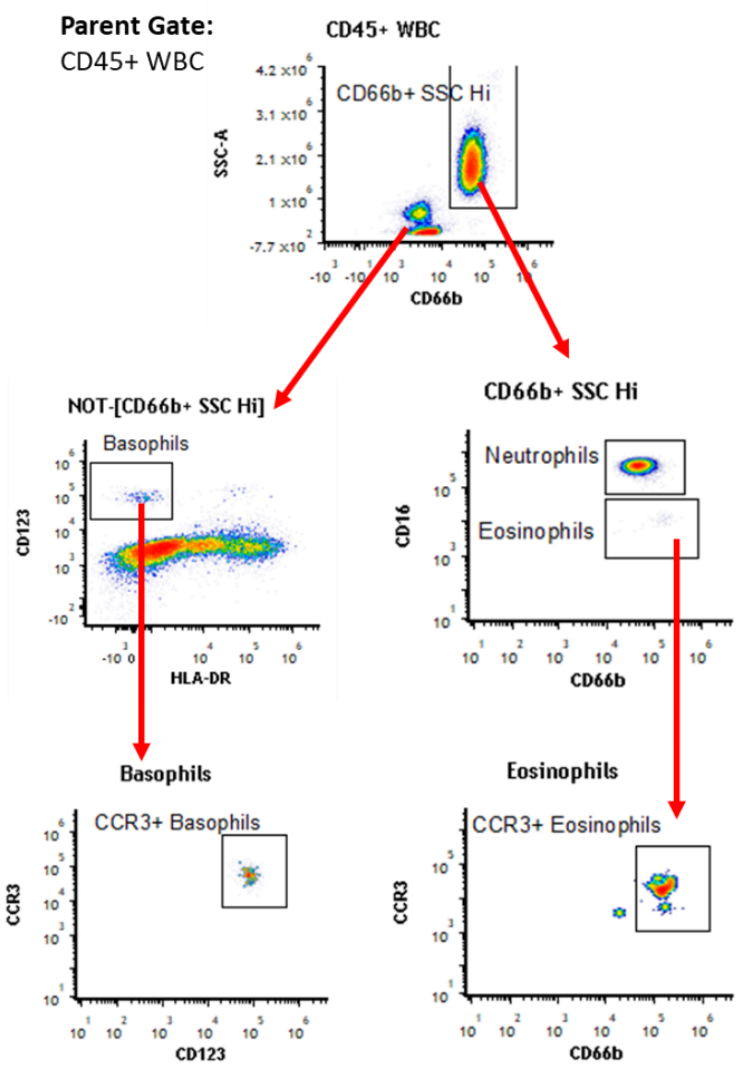

Figure 3: Granulocyte Gating Strategy. CD45+ leukocytes were gated based on CD66b+ SSC Hi events, with a NOT gate used to define all other events within the CD45+ compartment. Within the CD66b+ SSC Hi gate, CD16 high events were identified as neutrophils, while CD16- events were identified as eosinophils. Eosinophils were further assessed for CCR3 expression. From the NOT-[CD66b+ SSC Hi] gate, basophils were identified by their expression of CD123 and lack of HLA-DR expression. A NOT-[Basophils] population was also defined for further analysis (Figure 4). Basophils were additionally assessed for CCR3 expression.

Figure 4

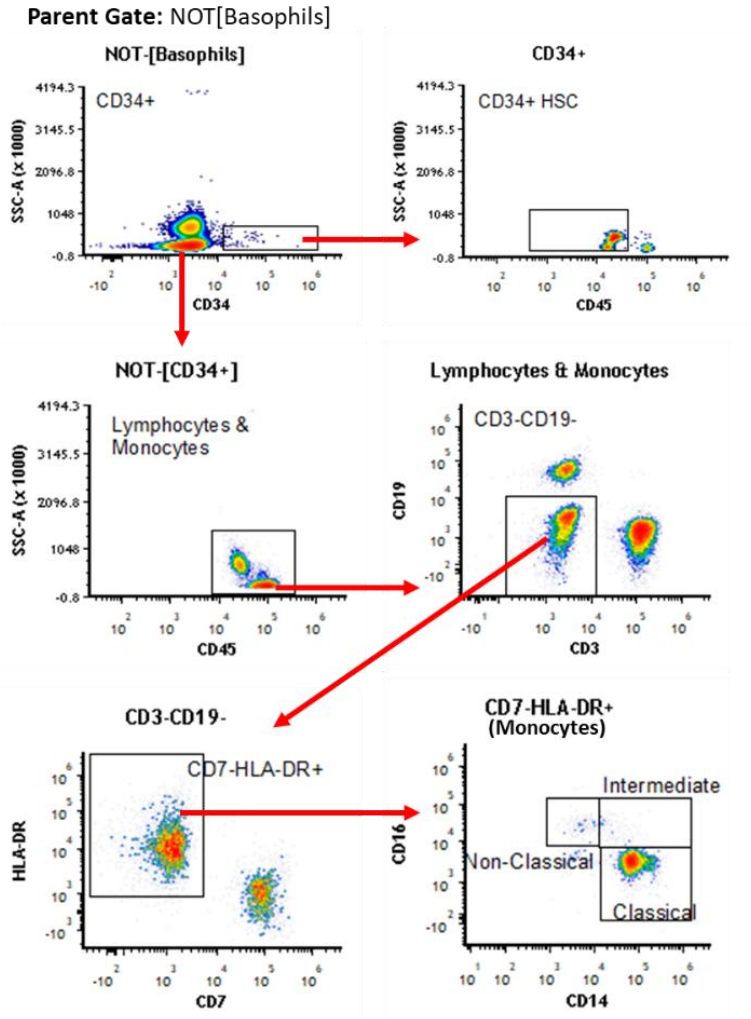

Figure 4: HSC and Monocyte Gating. CD34+ events were identified within the NOT-[Basophils] population (Figure 3). Additionally, a NOT[CD34+] population was defined for further analysis. From the CD34+ gate, CD34+ HSCs were identified based on dim expression of CD45. From the NOT-[CD34+] population, lymphocytes and monocytes were defined as CD45+ and low-to-medium SSC-A. CD3- CD19- events were gated from within the lymphocyte and monocyte gate, and a NOT-[CD3- CD19-] population was defined for further analysis (Figure 5). CD7- HLA-DR+ events were gated within the CD3-CD19- population, and a NOT-[CD7- HLA-DR+] population was defined for further analysis (Figure 4). Within the CD7- HLA-DR+ gate, the expression of CD14 and CD16 was used to identify classical (CD14+ CD16-), non-classical (CD14- CD16+), and intermediate (CD14+ CD16+) monocyte populations.

Figure 5

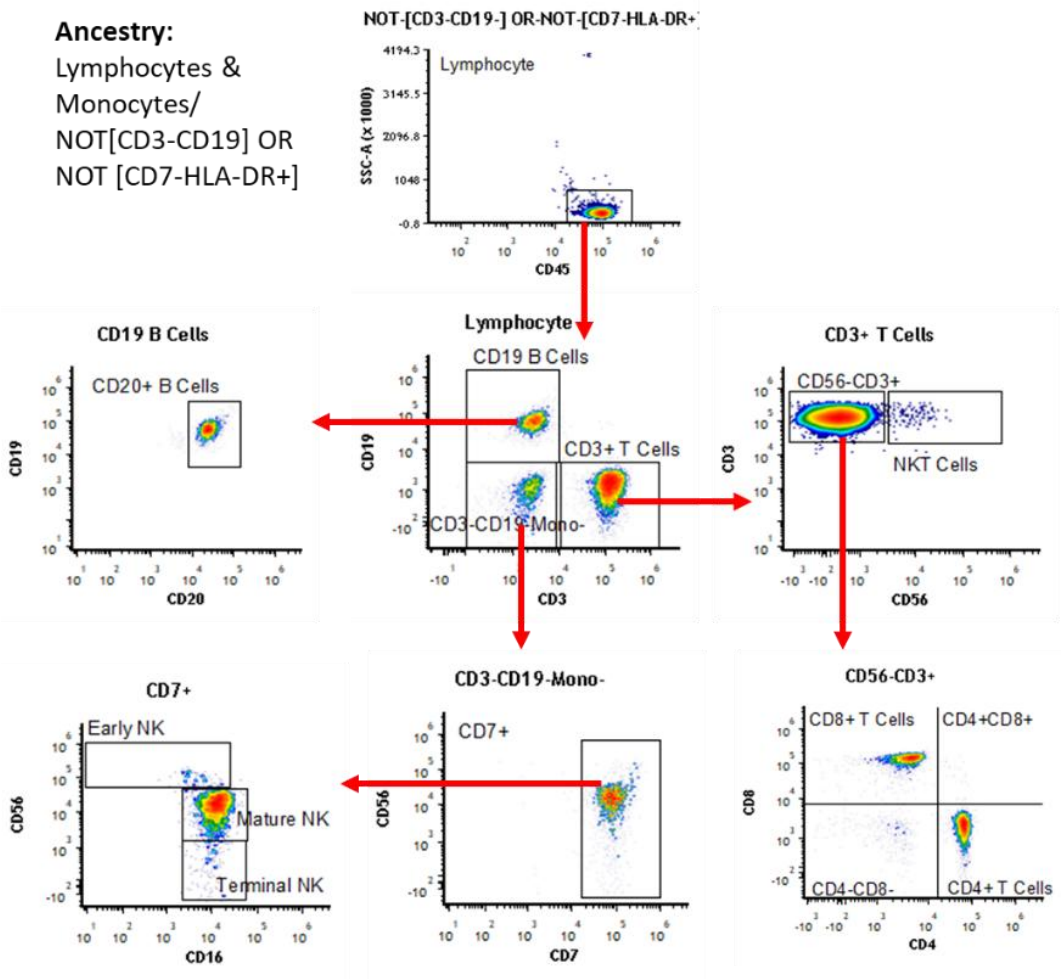

Figure 5: Lymphocyte Gating. Within events defined as NOT-[CD3- CD19-] OR NOT-[CD7- HLA-DR+] (see Figure 4), CD45-bright, SSC-A low events were identified as lymphocytes. Lymphocytes were gated into CD3- CD19+ B cell, CD3+ CD19- T cell, and CD3- CD19- populations. B cells were further assessed for expression of CD20. T cells were gated into CD56+ NK T cells and CD56- CD3+ T cells. CD56- CD3+ T cells were gated into CD4+ CD8- (CD4+ T cells), CD4- CD8+ (CD8+ T cells), CD4+ CD8+, and CD4- CD8-. CD7+ events were gated within the CD3- CD19- population. Within the CD7+ gate, CD16 and CD56 were used to identify early NK (CD16 dim or negative, CD56 bright), mature NK (CD16+, CD56 mid), and terminal NK (CD16+, CD56 dim) populations.

Figure 6

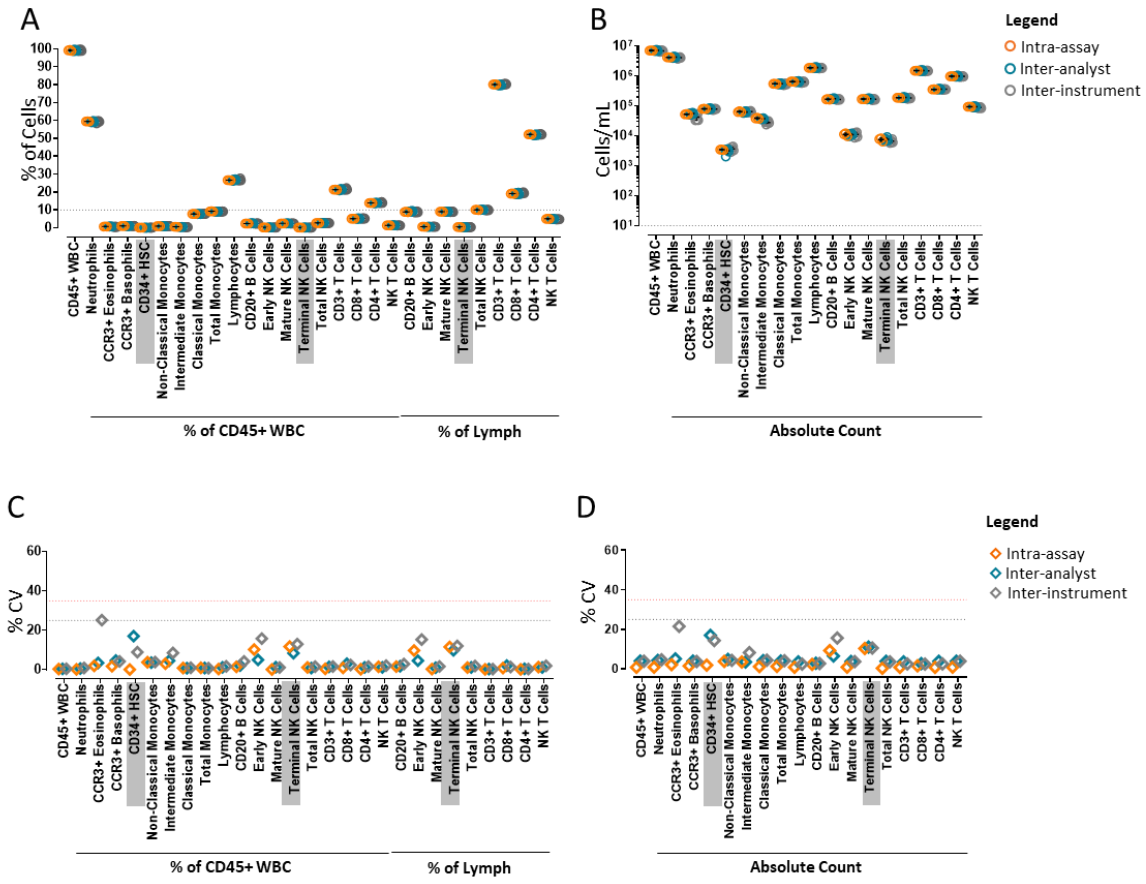

Figure 6: Validation results at Site 1. A) Percentage of cells measured for each identified population (x-axis) relative to the total CD45+ WBC (% of CD45+ WBC) and the total lymphocytes (% of Lymph) for lymphocyte populations. **B)** Absolute count of cells for each identified population (x-axis), reported as cells/mL of whole blood. Absolute counts were measured in each replicate of the validation assays. Error bars in A and B represent the mean with standard deviation among replicates of the same validation assay. **C-D)** Mean % CV for each readout, shown for both frequency (C) and absolute count (D). Data are presented for each validation assay as indicated in the legend. Readouts with insufficient numbers of gated events (fewer than 100) are highlighted in grey.

Figure 7

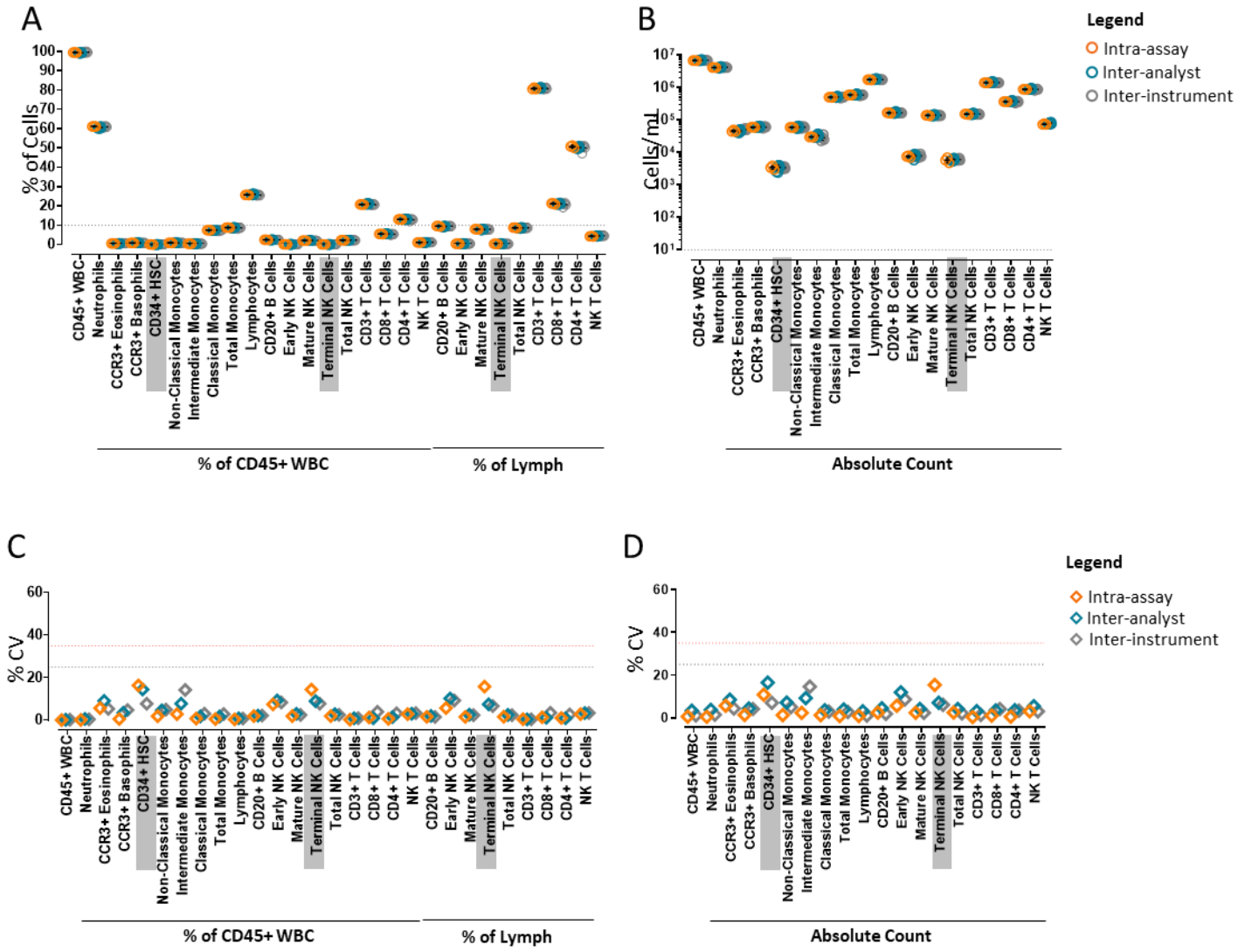

Figure 7: Validation results at Site 2. A) Percentage of cells measured for each identified population (x-axis) relative to the total CD45+ WBC (% of CD45+ WBC) and the total lymphocytes (% of Lymph) for lymphocyte populations. **B)** Absolute count of cells for each identified population (x-axis), reported as cells/mL of whole blood. Absolute counts were measured in each replicate of the validation assays. Error bars in A and B represent the mean with standard deviation among replicates of the same validation assay. **C-D)** Mean % CV for each readout, shown for both frequency (C) and absolute count (D). Data are presented for each validation assay as indicated in the legend. Readouts with insufficient numbers of gated events (fewer than 100) are highlighted in grey.

Figure 8

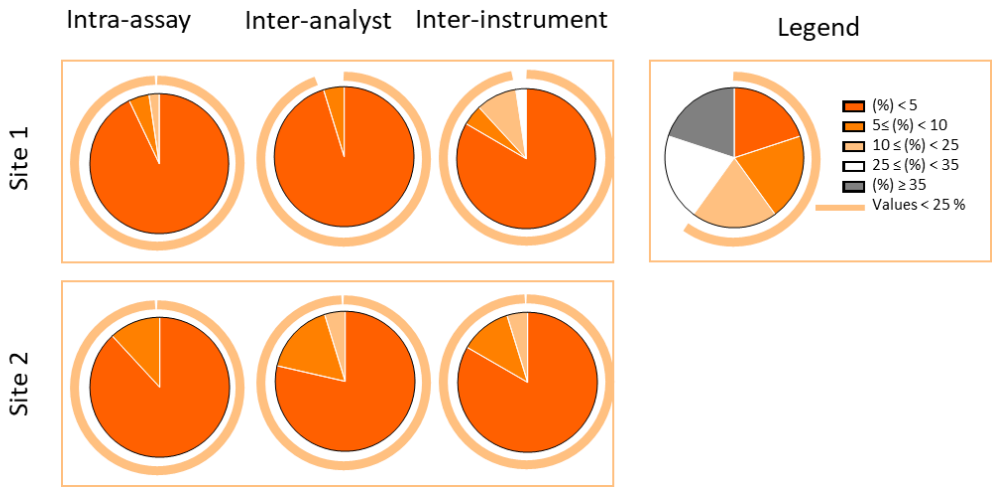

Figure 8: Precision assessment at Site 1 and Site 2. Pie charts for each validation assay, illustrating the proportion of readouts categorized into five levels of mean % CV measured at Site 1 and Site 2. The light orange line highlights values of % CV below 25%. Readouts with insufficient gated events (fewer than 100) are excluded from this analysis.

Figure 9

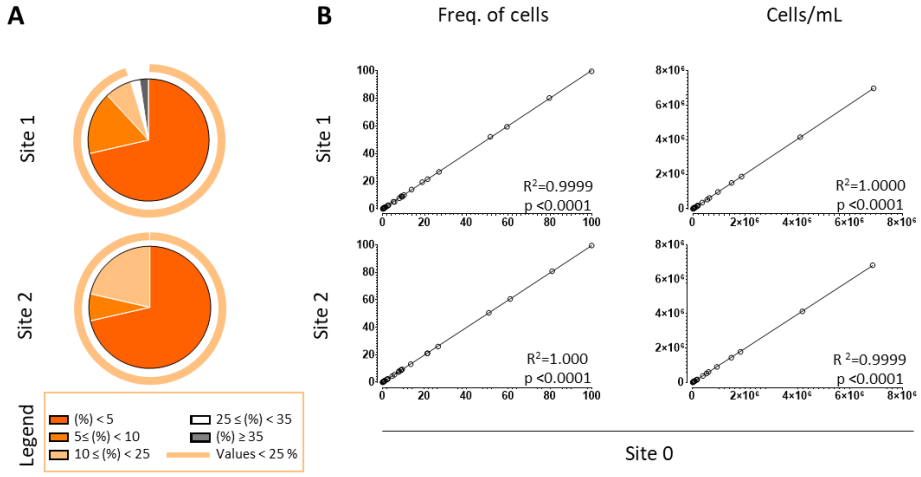

Figure 9: Coss-Site Reproducibility Assessment. A) Pie charts for cross-site assessment at Site 1 and at Site 2, illustrating the proportion of readouts categorized into five levels of mean % CV measured between Site 0 and Site 1, and between Site 0 and Site 2. The light orange line highlights values of % CV below 25%. Readouts with insufficient gated events (fewer than 100) are excluded from this analysis. **B)** A scatter plot illustrating the relationship between redouts (Frequency of cells and Cells/mL) obtained at Site 0 [X-Axis] and Site 1 or Site 2 [Y-axis]. Each data point represents an individual measurement. The solid line represents the Pearson regression fit. The Pearson correlation coefficient (R^2) and corresponding p-value are displayed, demonstrating the strength and significance of the linear association between the two variables.

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