

Review Article**Cytofluorometric Methods for Assessing Absolute Numbers of Cell Subsets in Blood**

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The enumeration of absolute levels of cells and their subsets in clinical samples is of primary importance in HIV+ individuals (CD4+ T lymphocyte enumeration), in patients who are candidates for auto-transplantation (CD34+ hematopoietic progenitor cells) and in evaluating leucoreduced blood products (Residual white blood cells). These measurements share a number of technical options, namely single- or multiple-color cell staining and logical gating strategies. These can be accomplished using single- or dual- platform counting technologies employing cytometric methods. Dual-platform counting technologies couple the percentage of positive cell subsets obtained by cytometry and the absolute cell count obtained by automated hematology analyzers to derive the absolute value of such subsets. Despite this approach having many conceptual and technical limitations, it is traditionally considered as the reference method for absolute cell count enumeration. As a result, the development of single-platform technologies has recently attracted attention with several different technical approaches now being readily available. These single-platform approaches have less sources of variability and a number of reports now clearly demonstrate that they provide better CV's in multicenter studies and a lower chance to generate aberrant results. These methods are therefore candidates for the new gold standard for absolute cell assessments. The currently available technical options are discussed in this review together with the results of some cross-comparative studies. Each analytical system has its own specific requirements as far as the dispensing precision steps are concerned. The importance of precision reverse pipetting is emphasized. Issues still under development include the establishment of the critical error ranges, which are different in each test setting and the applicability of simplified low-cost techniques to be used in countries with limited resources. *Cytometry (Comm. Clin. Cytometry)* 42:327-346, 2000. © 2000 Wiley-Liss, Inc.

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