

Claims:

1. A method for validating measurements by a fluorescence-based analytical instrument of a stained target cell population in a test sample, the method comprising:
 - a. negatively staining or having negatively stained cells in a first portion of a reference sample comprising target cells expressing a target cell marker;
 - b. positively staining or having positively stained the target cells in a second portion of the reference sample;
 - c. running the reference sample first portion through the instrument to obtain a fluorescence measurement indicative of the target cell concentration in the reference sample first portion, and running the reference sample second portion through the instrument to obtain a fluorescence measurement indicative of the target cell concentration in the reference sample second portion;
 - d. based on the fluorescence measurements obtained in (c), preparing a dilution series comprising a plurality of dilution samples, each dilution sample having a nominal concentration of the target cells, each nominal cell concentration greater than the concentration of target cells indicated by the fluorescence measurement of the negatively stained first portion in (a), wherein the nominal concentration in each dilution sample differs from the nominal concentration in each of the remaining dilution samples;
 - e. running the series of dilution samples from (d) through the instrument to obtain a series of fluorescence measurements comprising a fluorescence measurement for each a dilution sample;
 - f. for each dilution sample, comparing the nominal cell concentration from (d) and the fluorescence measurement from (e) to quantify the performance of the staining method in the instrument.
2. The method of claim 1, wherein comparing in (f) comprises performing a statistical calculation on the difference between the nominal cell concentration and the fluorescence measurement for each dilution sample to determine at least

one of linearity, range, accuracy, precision, limit of detection (LOD) and lowest limit of quantification (LLOQ) for the instrument and staining method.

3. The method of claim 1, wherein negatively staining the target cells in the reference sample first portion in (a) comprises introducing to the reference sample first portion (i) a fluorescent dead cell exclusion dye, (ii) a non-specific antibody conjugated to a fluorochrome, and (iii) a specific antibody capable of specifically binding a target marker on the target cell.
4. The method of claim 3, wherein positively staining the target cells in cells in the reference sample second portion in (b) comprises introducing to the reference sample second portion (i) the fluorescent dead cell exclusion dye, and (ii) and the specific antibody of claim 3 conjugated to the fluorochrome of claim 3.
5. The method of claim 3 or 4, wherein the fluorescent dead cell exclusion dye is selected from a nucleic acid binding dye, propidium iodide, DAPI, DRAQ7, 7-AAD, TO-PRO-3, and an amine-reactive dye.
6. The method of claim 3, wherein the fluorochrome-conjugated non-specific antibody comprises an antibody lacking the capability of specifically binding to a cell antigen.
7. The method of claim 7, wherein the fluorochrome-conjugated non-specific antibody comprises any one of IgD, IgG, IgA, IgM or IgE conjugated to the fluorochrome.
8. The method of any one of claims 3-8, wherein the fluorochrome is selected from Allophycocyanin (APC), APC C750, APC AF700, brilliant violet (BV)421, BV510, hilite 7 (H7) BV605, BV650, PE CF594, Fluorescein isothiocyanate (FITC), R-Phycoerythrin (PE or R-PE), PE-Cy7 (PE coupled to cyanine dye Cy7), APC-Cy7 (APC coupled to cyanine dye Cy7), APC-H7 (APC coupled to the Cy analogue Hilite 7 (H7)).

9. The method of any preceding claim, wherein the target cell marker is selected from CD3 as a T-cell marker, CD19 as a B-cell marker, CD235a as an erythrocyte marker, CD56 as a Natural Killer (NK) cell marker, CD14 as a monocyte marker, and CD66b as a granulocyte marker.
10. The method of claim 1, wherein the calculating in (f) is performed by a processor coupled with the instrument.
11. The method of claim 2, wherein determining the LLOQ comprises identifying the concentration of target cells associated with a predetermined criterion for precision and a predetermined criterion for accuracy.
12. The method of claim 1, wherein the test sample comprises the target cell present in a concentration of no more than 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, or 0.02%.
13. The method of claim 12, wherein the dilution series comprises a dilution sample having a highest concentration of the target cells, wherein the highest concentration is 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, or 0.02%.
14. The method of claim 4, wherein the negatively stained first portion of the test sample is prepared in the absence of a cell-depletion step.
15. The method of claim 1, wherein the fluorescence-based instrument is a flow cytometer.
16. A method for validating measurements by a flow cytometer of a stained target cell population in a test sample, the method comprising:
 - a. negatively staining or having negatively stained cells in a first portion of a reference sample comprising target cells expressing a target cell marker;
 - b. positively staining or having positively stained the target cells in a second portion of the reference sample;
 - c. running both the reference sample first portion and second portion through the flow cytometer to obtain fluorescence measurements

- indicative of the target cell concentration in each of the reference sample first portion and the reference sample second portion;
- d. based on the fluorescence measurements obtained in (c), preparing a series of dilution samples each having a nominal concentration of the target cells varying systematically across the dilution series, wherein the nominal cell concentration of at least one dilution sample is greater than the concentration of target cells indicated by the fluorescence measurement of the negatively stained first portion in (a);
 - e. running the series of dilution samples from (d) through the flow cytometer to obtain a series of fluorescence measurements comprising a fluorescence measurement for each a dilution sample;
 - f. for each dilution sample, comparing the nominal cell concentration from (d) and the fluorescence measurement from (e) to quantify the performance of the staining method in the flow cytometer.
17. The method of claim 16, wherein comparing in (f) comprises performing a statistical calculation on the difference between the nominal cell concentration and the fluorescence measurement for each dilution sample to determine at least one of linearity, range, accuracy, precision, limit of detection (LOD), and lowest limit of quantification (LLOQ) for the instrument and staining method.
18. The method of claim 16, wherein negatively staining the target cells in the reference sample first portion in (a) comprises introducing to the reference sample first portion (i) a fluorescent dead cell exclusion dye, (ii) a non-specific antibody conjugated to a fluorochrome, and (iii) a specific antibody capable of specifically binding a target marker on the target cell, unconjugated to the fluorochrome.
19. The method of claim 18, wherein positively staining the target cells in cells in the reference sample second portion in (b) comprises introducing to the reference sample second portion (i) the fluorescent dead cell exclusion dye, and (ii) and the specific antibody of claim 18 conjugated to the fluorochrome of claim 18.

20. The method of claim 18 or 19, wherein the fluorescent dead cell exclusion dye is selected from nucleic acid binding dye, propidium iodide, DAPI, DRAQ7, 7-AAD, TO-PRO-3, and an amine-reactive dye.
21. The method of claim 18, wherein the non-specific fluorochrome-conjugated antibody comprises an antibody lacking the capability of specifically binding to a cell antigen.
22. The method of claim 21, wherein the the non-specific fluorochrome-conjugated antibody comprises any one of IgD, IgG, IgA, IgM or IgE conjugated to the fluorochrome.
23. The method of any one of claims 18-22, wherein the fluorochrome is selected from Allophycocyanin (APC), APC C750, APC AF700, brilliant violet (BV)421, BV510, hilite 7 (H7) BV605, BV650, PE CF594, Fluorescein isothiocyanate (FITC), R-Phycoerythrin (PE or R-PE), PE-Cy7 (PE coupled to cyanine dye Cy7), APC-Cy7 (APC coupled to cyanine dye Cy7), APC-H7 (APC coupled to the Cy analogue Hilite 7 (H7)).
24. The method of any of claims 16-23, wherein the target cell marker is selected from CD3 as a T-cell marker, CD19 as a B-cell marker, CD235a as an erythrocyte marker, CD56 as a Natural Killer (NK) cell marker, CD14 as a monocyte marker, and CD66b as a granulocyte marker.
25. The method of any of claims 16-23, wherein the target cell population is a T cell population, and the target cell marker is CD3.
26. The method of claim 16, wherein the calculating in (f) is performed by a processor coupled with the instrument.
27. The method of claim 17, wherein determining the LLOQ comprises identifying the concentration of target cells associated with a predetermined criterion for precision and a predetermined criterion for accuracy.

28. The method of claim 18, wherein the test sample comprises the target cell present in a concentration of no more than 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, or 0.02%.
29. The method of claim 28, wherein the dilution series comprises a dilution sample having a highest concentration of the target cells, wherein the highest concentration is 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, or 0.02%.
30. The method of any preceding claim, wherein the series of dilution samples comprises at least two, three, four, five, six, seven, eight, nine or ten dilution samples.
31. A non-transitory computer-readable medium comprising instructions for a computer processor for performing the comparison in (f) according to claim 1 or claim 16, or for performing the statistical calculation according to claim 2, 12, 17 or 27.
32. A system for validating fluorescence measurements in a test sample made by a fluorescence-based instrument, the system comprising: the fluorescence-based instrument; and a computer coupled with the fluorescence-based instrument and comprising the computer readable medium of claim 31.
33. The system of claim 32, wherein the fluorescence-based instrument is a flow cytometer.
34. A kit comprising reagents for staining cells in a reference sample for validating a fluorescence-based analytical method for analyzing target cells in a test sample, the kit comprising:
- (i) negative stain reagents comprising: (a) a fluorescent dead cell exclusion dye, (b) a non-specific antibody conjugated to a fluorochrome, and (c) a specific antibody capable of specifically binding a target marker on the target cells, unconjugated to the fluorochrome;

- (ii) positive staining reagents comprising: (a) the fluorescent dead cell exclusion dye, and (b) the specific antibody conjugated to the fluorochrome; and
- (iii) instructions for (a) negatively staining a first portion of the reference sample, (b) positively staining a second portion of the reference sample, and (c) preparing a dilution series comprising a plurality of dilution samples each having a nominal concentration of the target cells varying across the dilution series, wherein the nominal cell concentration of at least one dilution sample is greater than the concentration of target cells indicated by fluorescence measurement of the negatively stained first portion.