

[0010] In addition, AQUIOS STEM System and the methods described herein provide analysis options that are adapted to individual regulatory requirements, such as the International Society for Hematotherapy and Graft Engineering (ISHAGE) Guidelines for CD34+ enumeration and the European Pharmacopoeia, that differ in their request for duplicate runs and the use of a negative control. This flexibility also considers the parallel enumeration of CD3+ T cells together with the CD34+ stem and progenitor cell population, in that the AQUIOS STEM System provides a total of six (6) analysis options to choose from as part of the IVD solution. This is unique to the market and eliminates the need for time-consuming self-validation of lab-developed tests.

[0011] The level of automation provided by the AQUIOS STEM System is achieved by using pre-mixed and ready-to-use reagent combinations adapted for automation whilst ensuring the highest level of data traceability. Currently available manual test kits use solutions for red blood cell lysis (an essential step in sample preparation) that require manual daily preparation from a concentrate, and that negatively affect cell viability so that samples need to be stored on ice before the actual analysis. In contrast, AQUIOS STEM System uses a gentler red blood cell lysing reagent that is ready to use and can be used at room temperature, which allows automation of CD34+ and CD3+ counts in a way known for other automated flow cytometry applications, but not for stem cell enumeration. All reagent vials critical for the test are barcoded with individual identifiers and essential quality control parameters such as reagent type, reagent lot, day of first use, expiration date, etc., and stored in one database together with the sample information, providing the level of data traceability mandated by today's accreditation authorities.

[0012] AQUIOS STEM System is validated in clinical studies against its predicate method, the Stem-Kit for the FC500 flow cytometer, that is perceived as the "gold standard" in the market for clinical CD34+ enumeration and that was also used as reference for the manual CD34+ enumeration solutions of other manufacturers. With the AQUIOS STEM System, the "gold standard" is taken to the next level by providing a unique new solution for the enumeration of CD34+ stem and progenitor cells that takes away the burden of stem cell enumeration from clinical laboratories.

[0012a] In one aspect, the present disclosure refers to an automated flow cytometric method for the analysis and enumeration of at least one of hematopoietic stem cells, hematopoietic progenitor cells, and T-cells, the method comprising:

placing one or more blood samples into one or more receptacles of a sample
 plate located in a sample preparation area of a flow cytometer;
 treating the one or more receptacles with at least one reagent to obtain at least
 one first composition, at least one of the at least one reagent being at least one
 imaging reagent comprising recognition elements specific for markers on at least
 one of the hematopoietic stem cells, the hematopoietic progenitor cells, and T-
 cells;
 incubating the at least one first composition for a time period sufficient for binding
 of the at least one imaging reagent comprising recognition elements specific for
 the markers on the at least one of the hematopoietic stem cells, hematopoietic
 progenitor cells, and T-cells to give at least one second composition;
 treating the at least one second composition with a lysing reagent to give at least
 one third composition; and
 analyzing the at least one third composition by flow cytometry to obtain at least
 one of a viable and total hematopoietic stem cell count viable and total progenitor
 cell count, and viable and total T-cell count in the one or more blood samples.
[0012b] In one aspect, the present disclosure refers to an automated flow
 cytometric method for the analysis and enumeration of at least one of
 hematopoietic stem cells, hematopoietic progenitor cells, and T-cells, the method
 comprising:
 placing a first blood sample into a first receptacle of the sample plate;
 treating the first receptacle with at least one reagent to obtain a first composition
 f^1 and incubating first composition f^1 to give second composition s^1 , at least one
 of the at least one reagent being at least one imaging reagent comprising
 recognition elements specific for markers on at least one of the hematopoietic
 stem cells, hematopoietic progenitor cells, and T-cells;
 while first composition f^1 is incubating, placing a second blood sample into a
 second receptacle of the sample plate and treating the second receptacle with at
 least one reagent, at least one of the at least one reagent being at least one
 imaging reagent comprising recognition elements specific for markers on at least
 one of hematopoietic stem cells, hematopoietic progenitor cells, and T-cells, to
 obtain a first composition f^2 and incubating first composition f^2 to give second
 composition s^2 ;
 treating second compositions s^1 and s^2 with a lysing reagent to give third
 compositions t^1 and t^2 ; and
 analyzing third compositions t^1 and t^2 by flow cytometry to obtain at least one of a
 viable and total hematopoietic stem cell count, viable and total progenitor cell
 count, and viable and total T-cell count.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] These and other features, aspects, and advantages of the present technology will become better understood with regard to the following drawings:

[0014] FIG. 1A is a checklist of the characteristics of a solution for the identification and quantification of CD34+ hematopoietic progenitor cells that addresses requirements of clinical laboratories.

5 **[0015]** FIG. 1B is a flow diagram of a method described herein for the analysis and enumeration of at least one of hematopoietic stem cells, hematopoietic progenitor cells, and T-cells.

[0016] FIG. 2 is a perspective view of one example of a diagnostic instrument, wherein the instrument is shown coupled with a specimen autoloader and includes a flow cytometer.

10 **[0017]** FIG. 3 is an enlarged perspective view of a portion of the diagnostic instrument shown in FIG. 2.

[0018] FIG. 4 is a front perspective view of the diagnostic instrument of FIGS. 2-3, showing the instrument during operation.

15 **[0019]** FIG. 5 is an enlarged view of the portion of the diagnostic instrument that is capable of sampling a single specimen tube at a time.

[0020] FIG. 6 is a front perspective view of the external housing of the proposed diagnostic instrument shown in FIGS. 2-5.

20 **[0021]** FIG. 7 is a front perspective view of the external housing of another example, in which the specimen autoloader is removed and specimen tubes are inserted through the front door.

[0022] FIG. 8 is an example of a software component system that can be used in the methods and systems described herein.

[0023] FIG. 9 is a table showing the panel options for the clinical quantification of CD34+ HPC, either with or without the analysis of residual T cells.

25 **[0024]** FIGS. 10A-10C are plots showing equivalence of the three CD34+ analysis options STEM Panel (tests in duplicate plus negative control), STEM Duplicate (tests in duplicate, no negative control) and STEM Single (single test) for CD34+ absolute counts (cells/ μ L). These three analysis options are for tests that do not include CD3.

30 **[0025]** FIGS. 11A-11C are plots showing the equivalence of the three CD34+ plus CD3+ analysis options STEM ALLO Panel (tests in duplicate plus negative control), STEM ALLO Duplicate (tests in duplicate, no negative control) and STEM ALLO Single (single test) for CD34+ absolute counts (cells/ μ L). These three analysis options are for tests that do include CD3.

35 **[0026]** FIGS. 12A-12C are plots showing the equivalence of the three CD34+ plus CD3+ analysis options STEM ALLO Panel (tests in duplicate plus negative control), STEM ALLO Duplicate (tests in duplicate, no negative control) and STEM

ALLO Single (single test) for CD3+ absolute counts (cells/ μ L). These three analysis options are for tests that do include CD3.

[0027] FIG. 13 is a representative manual flow cytometry workflow, as described in Example 2.

5 **[0028]** FIG. 14 is a representative AQUIOS CL Flow Cytometry System workflow, as described Example 2.

[0029] FIGS. 15A-15B are plots of sample processing turnaround time and operator hands-on time for one CD34+ sample or a batch of 10 CD34+ samples on the FC500 with Stem-Kit (Alternative Method, empty bars) and AQUIOS STEM System (AQUIOS, hashed bars).

10 **[0030]** FIGS. 16A-16B are plots of process time and operator hands-on time for Quality Control (QC) procedures on the FC500 with Stem-Kit (Alternative Method, grey bars) and AQUIOS STEM System (AQUIOS, red bars) per workday (FIG. 16A) and a 5-day work week (FIG. 16B).

15 DESCRIPTION

[0031] Reference will now be made in detail to certain embodiments of the disclosed subject matter. While the disclosed subject matter will be described in conjunction with the enumerated claims, it will be understood that the exemplified subject matter is not intended to limit the claims to the disclosed subject matter.

20 **[0032]** With the increased use of mobilized, peripheral stem and progenitor cells (PBSC) for transplantation purposes, researchers, in concert with the International Society for Hematotherapy and Graft Engineering (ISHAGE), described in 1996 a set of standards for CD34+ enumeration with the intent to provide a simple, sensitive method that allows for a high degree of accuracy and interlaboratory reproducibility. The resulting "ISHAGE Guidelines" soon became the gold standard for the enumeration of hematopoietic CD34+ progenitor cells by flow cytometry. In 25 1998, a research group published a modified version of the 1996 guidelines, by introducing beads for absolute counting, adding 7-aminoactinomycin D (7-AAD) as viability dye to exclude dead cells, and a lysing reagent lacking fixatives, such as formaldehyde. These modifications converted the basic protocol into a single-platform method, and the resulting "Single platform with viability dye ISHAGE Guidelines" are still, more than 20 years later, mostly untouched.

30 **[0033]** The hallmark of the ISHAGE Guidelines is a sequential gating strategy that derives the number of CD34+ cells from viable leukocytes. The Guidelines also require tests to be run in duplicate together with a negative control in order to correct for testing variability and nonspecific cell and fluorochrome binding.

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