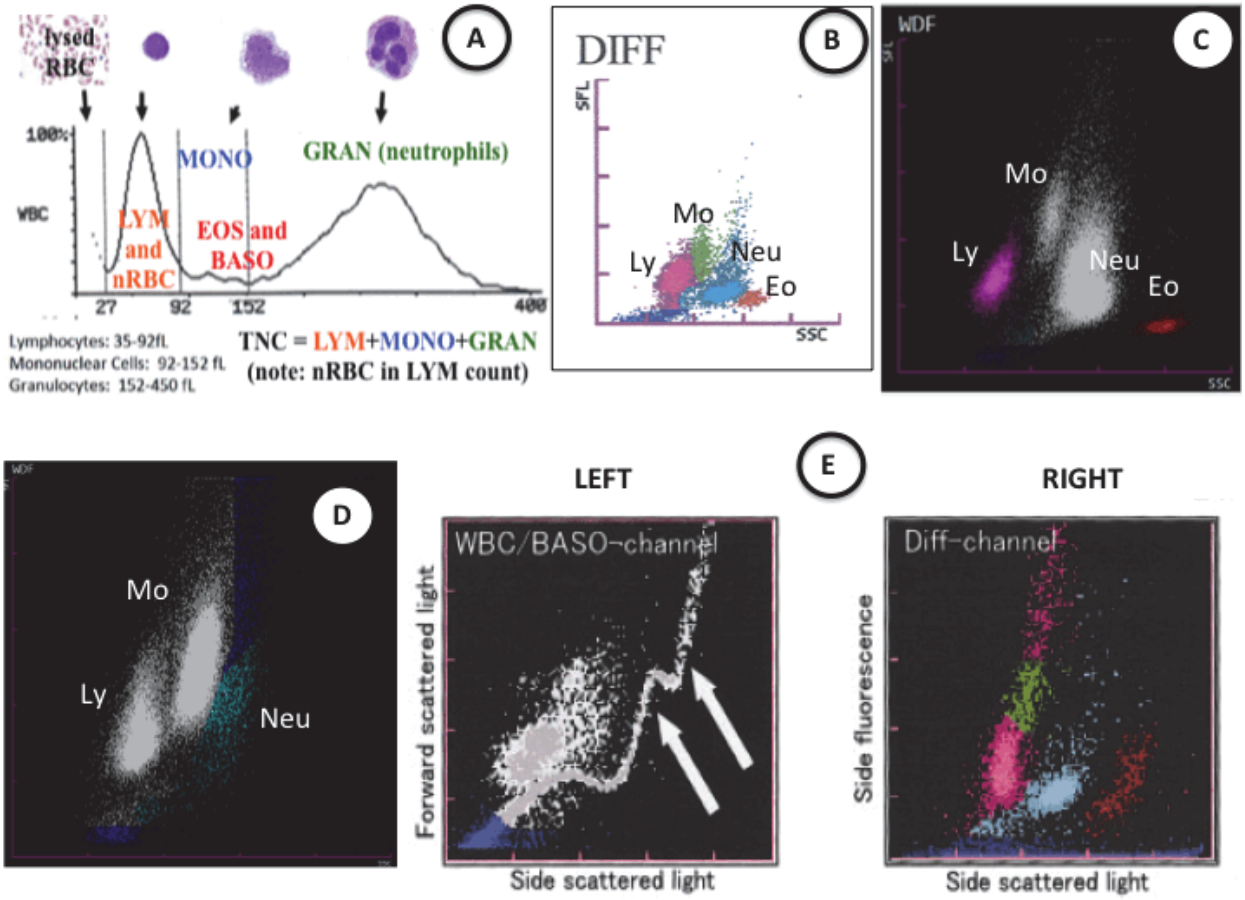

Figure 53-1. Sample data illustrating leukocyte cell differential results obtained by analysis of peripheral blood (A), cord blood [HPC(CB)] (B), growth factor-mobilized peripheral blood (C), apheresis collection of peripheral blood from panel C [HPC(A)] (D), and bone marrow [HPC(M)] (E) using an automated hematology analyzer. Panel A is a representative result obtained by electrical impedance-based cell counting providing a three-part leukocyte differential. Panels B through E represent results obtained by analysis of optical signals generated by semiconductor laser flow cytometry (Sysmex XE and XN series), which provides a five-part leukocyte differential and separate counting of nucleated red blood cells (NRBCs) (not shown). Panel E adapted from Shibata et al.[9]
BASO = basophils; EOS = eosinophils; LYM = lymphocytes; MONO = monocytes; TNC = total nucleated cell; WBC = white blood cell.

Some examples of these analyzers are the following:

1. Coulter LH series autoanalyzers employ "volume/conductivity/scatter (VCS) technology" to perform a five-part WBC differential achieved by superimposing an RF signal (to assess cellular complexity by measuring RF conductance) on the DC voltage applied to the electrodes of a conventional DC impedance detector (to measure cell volume). The detector aperture is placed in the path of a laser light source to simultaneously generate an optical scatter signal producing three simultaneous VCS signals for cell classification.
2. The Abbott CELL DYN series (Abbott, Abbott Park, IL) performs simultaneous measurements of multiple FSC and SSC signals with unpolarized and polarized laser light in an approach that is called multi-angle polarized side scatter (MAPSS) cell analysis.

3. The Siemens ADVIA system (Siemens, Munich, Germany) utilizes FSC for cell size (volume) estimation combined with light absorption measurements after staining cells to detect peroxidase activity [high in granulocytes (neutrophils and eosinophils), intermediate in monocytes, and low in lymphocytes].
4. The Sysmex XE and XN series (Sysmex, Kobe, Japan) employ FSC, SSC, and (side) fluorescence staining with a polymethine dye for WBC cell differentiation. Figure 53-1 (B through E) provides examples of the results typically obtained by optical analysis of HPC(CB) (B), growth factor-mobilized peripheral blood (C), HPC(A) (D), and HPC(M) (E) by using the Sysmex XE and XN series autoanalyzers.

Both the ADVIA and Sysmex XE and XN series devices separately quantify total WBCs and basophils by light scatter measurements after selective detergent lysis of RBCs and surfactant modification of other WBC populations while permitting relative preservation of basophils.

As shown in Fig 53-1(B), the (side) fluorescence (SFL) and side scatter (SSC) optical signals generated by analysis of HPC(CB), which has a cellular composition that most closely resembles peripheral blood from healthy individuals, produces readily identifiable separate cell clusters for the lymphocyte (Ly), monocyte (Mo), and granulocyte (Gran) [neutrophils (Neu) and eosinophils (Eo)] populations. On the Sysmex XE and XN series, basophils and NRBCs are counted in separate channels, and the reported WBC count does not include NRBCs when they are analyzed separately. In addition, the cell counts are accurate and do not require further confirmation by smear review as long as there are no interpretive program messages or "flagged results" indicating the possibility of abnormalities in cell morphology or the presence of significant numbers of abnormal cells.

For growth factor-mobilized peripheral blood before apheresis collection [Fig 53-1(C)] the Mo and Neu cell clusters are often "abnormal," most likely because of the presence of immature monocytes and granulocytes after growth factor [granulocyte-colony-stimulating factor (G-CSF)] mobilization. When adoptive cluster analysis identifies abnormalities in cell cluster location and shape, it is depicted by the gray coloring of the corresponding regions [ie, the cell clusters are grayed-out, as shown for Mo and Neu in Fig 53-1(C)]. Under these circumstances, results may be reported by the instrument for the corresponding cell populations, but are flagged to indicate that they may be unreliable. Often the data for the leukocyte differential are not displayed by the instrument, to prevent reporting results that are likely unreliable (inaccurate). However, the total WBC count is generally not flagged, which indicates that this result is judged to be reliable and accurate.

In our experience with HPC(A) [Fig 53-1(D)], the cell clusters in the DIFF or WDF channel are almost always grayed-out and accompanied by interpretive program messages indicating the presence of an abnormal WBC scattergram with atypical lymphs, blasts, immature granulocytes, "left shift," and other abnormal or suspect flags. Although the results are displayed in gray, it is still possible to appreciate the enrichment of lymphocytes and monocytes and the depletion of granulocytes that is generally seen in HPC(A), compared to growth factor-mobilized peripheral blood (compare Panels C and D in Fig 53-1). We routinely dilute HPC(A) products 1:5 before analysis to improve cell discrimination in the DIFF or WDF channel. Total WBC results are reliable in the great majority of cases. However, results reported for MNC (Ly and Mo) and granulocytes are virtually always flagged, indicating that they should be considered as an estimate only and that a smear review should be performed for an accurate granulocyte and MNC count.

HPC(M) are the most challenging [Fig 53-1(E)] products to analyze on an automated hematology analyzer. With the ratio of myeloid to erythroid cells in "normal" BM aspirate in the range of 2-3 to 1, up to one-third of the TNCs in HPC(M) are erythroblasts (mature and immature NRBCs). The early pronormoblasts and basophilic normoblasts most likely appear in cell clusters with other blast cells, and they usually comprise <5% of TNCs in "normal" marrow. The intermediate and late stages of RBC maturation, including mature NRBCs, most closely resemble Ly and are included in the Ly count as has been reported previously.[7-9] Besides the high content of NRBCs, BM contains fat particles that can interfere with cell counting. For the Sysmex XE series, the presence of lipid particles in the WBC/BASO scattergram [indicated by the two white arrows in Fig 53-1(E), LEFT] leads to overestimation of the total WBC.[8,9] It is interesting that lipid

particles are not clearly evident in the DIFF scattergram for HPC(M) [Fig 53-1(E), RIGHT], in which the surfactant used in the DIFF channel reagent is different from the one used in the WBC/BASO channel. HPC(M) also shows an unusual pattern of cell clusters in the DIFF channel, a pattern not typically seen in other HCT products (compare Panel E RIGHT to Panels B-D in Fig 53-1), which likely represents the many erythroid and myeloid precursors in BM.

For samples in which cell clusters are grayed-out and results for the cell differential are not reported directly by the instrument, the software may offer other means of determining the instrument's "best guess" for the WBC differential cell count. With suitable modification, the Sysmex XE series analyzers can provide accurate cell differentials in BM.[8,9] However, in the conventional configuration used by clinical labs, interference by lipid particles should be taken into consideration for HPC(M), which can easily be identified by close inspection of the WBC/BASO channel scattergram. Additional discussion of the challenges of analysis of HPC(M) can be found in Chapter 54 (Cell Counts and Differentials of Cellular Therapy Products), which also describes a procedure for removing the fat particles by centrifugation of the BM specimen.

Table 53-1 summarizes the types of automated hematology analyzers used by HCT laboratories participating in the College of American Pathologists (CAP) Stem Cell Processing external proficiency testing program. As shown in Table 53-1, the Sysmex XE and XN series are the most frequently used instruments capable of performing an automated five-part differential and separate NRBC count.

Table 53-1. Summary of Automated Hematology Analyzers Used for HSCT Product Characterization

Manufacturer	Model Number(s)	Technology for WBC Differential Count	Diff Type	% Labs
Abbott	CELL DYN 3700, CELL DYN SAPPHIRE	MAPSS (optical, multiangle polarized side scatter)	5-part + NRBC	4
Coulter	Z series, ACT DIF, ACT DIF2, MD2, ACT 8/10	DC impedance (cell volume)	3-part	20
Coulter	ACT 5diff	ACV (optical absorbance cytochemistry, volume by DC impedance)	5-part	3
Coulter	LH 750, LH 755, LH 780, LH 785	VCS (volume by DC impedance, radiofrequency conductivity, and optical side scatter)	5-part + NRBC	12
Siemens	ADVIA 120/2120	Optical histochemical staining (peroxidase activity) and optical scatter	5-part + NRBC	5
Sysmex	KX-21, KX-21N	DC impedance (cell volume)	3-part	6
Sysmex	XE-2100/2100D/L, XN Series	Optical forward and side scatter and "side" fluorescence after staining with polymethine dye	5-part + NRBC	19
Other	Not Specified	Not Specified	Not Specified	29

Data adapted from College of American Pathologists Stem Cell Processing Survey B, 2014. Data for hematology instrumentation were reported by a total of 94 participating laboratories.

Method 28-2. Optia MNC Collection

Department: Cell Therapy Apheresis	**Effective Date:**
Procedure Name: Optia MNC Collection	**Replaces Procedure Dated:**
Procedure Number:	**Retired:**

Scope:

CTA

Purpose/Principle:

Defines the process of performing the calculations and for the apheresis collection of HPC, MNC, and plasma for either autologous or allogeneic collections using the Spectra Optia.

Definition:

1. CTA – Cell Therapy Apheresis
2. HPC – Hematopoietic Progenitor Cells
3. MNC – Mononuclear cells including monocytes, lymphocytes and dendritic cells and HPC.
4. CTL – Cell Therapy Lab
5. SCVP – Infectious Diseases Testing (formerly Stem Cell Viral Panel)
6. PPE – Personal Protective Equipment
7. CVL – Central Venous Line
8. HAS – Hospital Approved Scrub
9. RPM – Revolutions per Minute
10. CIG/DNA – Cytoplasmic Immunoglobulin vs DNA
11. CTA Medical Director/Designee – Designee is a person with authority to act for the Facility Director and Medical Director
12. Transplant Team – Clinic Attending MD and nursing team
13. HLA – Human Leukocyte Antigen
14. EPF – Electronic Patient File
15. TC, Apheresis – Therapeutic Cells
16. NK – Natural Killer Cells
17. A/V F – Arterial/Venous Fistula
18. TBV – Total Blood Volume
19. WBC – White Blood Cell
20. Hct – Hematocrit
21. AIM – Automated Interface Management
22. RN – Registered Nurse
23. NA – Not Applicable

Equipment List/Reagents:

1. Spectra Optia with compatible blood warmer.
2. Functional CVL or venous access.
3. Spectra Optia collection set.
4. Appropriate labels.
5. Vital signs machine.
6. IV infusion pump.
7. Sampling Site Coupler.
8. ACD-A anticoagulant ×2.
9. Heparin 1000 units/mL.
10. Calcium Chloride 1 gm/10 mL (appropriate amount).
11. Aspirin 81 mg chewable (if needed).
12. IV tubing.
13. Normal Saline 250 mL.
14. Normal Saline 1000 mL × 2.
15. Needles: 18 gauge and alcohol swabs.
16. Syringes: 30 mL, 5 mL × 2, 10 mL × 2.
17. Appropriate lab tubes.
18. PPE.
19. Blood/fluid warmer set.
20. 3-way stopcock (male).
21. Bag access device with check valve and needle free port × 2.
22. 600 mL transfer bag (if concurrent plasma ordered).
23. CTA transport container (if needed).
24. CTA storage container.

Forms/Documents:

1. Clinical laboratories clinical flow cytometry request for cell therapy.
2. CTA lab requisition.
3. CTA collection orders.
4. CTA collection flow sheet.
5. CTA continuation nurses notes.
6. Blood bank requisition.
7. ACD-A/heparin reagent mixing log.
8. ACD-A/heparin label.
9. Completed CTA consult for collection and processing (prescription to collect).

10. For research protocols a completed copy of the donor consent.
11. Completed autologous donor testing form with required attachments.
12. Completed autologous health history questionnaire.
13. COBE cleaning log.
14. Appropriate patient consent forms and documentation for procedure.
15. CTA encounter form.
16. CTA physician's orders.
17. CTA progress notes.
18. CTA HPC disposition and release log.
19. CTA post collection apheresis instructions.

Specimen Required: N/A

Procedure:

I. Autologous Donor Selection
   A. Donor eligibility and suitability is performed by the Clinic Transplant Physician.
   B. CTA is informed by transplant coordinator of patient/donor's expected date of collection.
   C. A donor chart will be started in CTA to contain the following:
      1. Completed CTA consult for collection and processing.
      2. Copy of growth factor orders (if applicable).
      3. Copy of chemotherapy orders (if applicable).
      4. Completed autologous donor testing form with required attachments.
      5. Completed autologous health history questionnaire.
      6. CTA consultation form.
      7. Appropriate authorization and consent to surgical & medical procedures with collection information.
      8. CTA encounter form.
      9. CTA physician's orders.
      10. CTA collection orders.
      11. CTA collection flow sheet.
      12. Infectious disease testing results.
      13. Verification of CVL placement.
      14. Multidisciplinary patient education form.

II. Preprocedure Assessment
   A. Identify the patient at the beginning of each collection.
   B. Obtain informed consent for the collection.
   C. Compare the **Prescription to Collect** against the patient identification and review for signatures, total number of CD34 $\times 10^6$/kg cells to collect, need for, and amount of concurrent plasma, or any other pertinent information.
   D. All patients/donors will have a CVL placed prior to the collection. Accurate placement will be verified verbally or by written confirmation from Interventional Radiology, CVL Clinic, or Surgical Services. Verbal confirmation is recorded on the **CTA collection flow sheet** in the **Comments** section.
   E. Each day of collection an interim assessment of patient/donor suitability will be performed by the CTA staff. The assessment shall include at a minimum vital signs, review of pertinent lab data, review of current medications, changes in physical condition including but not limited to presence of peripheral edema and pain and documented on the **CTA collection flow sheet.**
   F. **If the patient/donor platelet count is ≥80, administer aspirin 81 mg to be chewed.**
   G. If any of the minimum requirements for collection are not met, CTA staff will contact transplant team for evaluation and follow-up until minimum requirements are met. Collection shall not proceed until all minimum requirements are met.
   H. Complete the **CTA collection flow sheet** with the following information:
      1. Procedure type, the name of the product collected, and the date.
      2. Procedure number—corresponding unique numeric identifier label.
      3. Directed recipient (mark "self").
      4. Expiration date (24 hours from end collection).
      5. Allergy history.
      6. CVL assessment and lab draw location.
      7. CVL dressing change per institutional procedure.
      8. Pain assessment.
      9. Patient sex, height, and weight in kg.
      10. Patient vital signs.

11. Record manufacturer, lot number, expiration date for all disposables, tubing, saline, ACD-A, reagents, and supplies.
12. Complete documentation for procedure as appropriate.
13. Document the procedure machine, blood warmer machine, IV pump and vital sign equipment numbers.
14. Assess the patient for any change in medications, condition, or symptoms and document in the "Comments" section of the flow sheet.

III. Machine Preparation
   A. ACD-A 1000 mL with 5,000 units heparin is the anticoagulant combination used for collection unless the donor has heparin allergy/antibody. For allergy/antibody, inform CTA Medical Director/designee for orders.
      1. Clean counter with HAS.
      2. Obtain the Heparin 1000 units/mL and verify the correct drug, dosage, and expiration date.
      3. Wash hands and gather all supplies.
      4. Wipe the top of the Heparin vial with alcohol pad.
      5. **Per** CTA Collection Orders **draw up the appropriate dose of heparin.**
      6. Wipe the infusion port of the ACD-A with alcohol pad.
      7. Inject correct dose of Heparin into the ACD-A bag.
      8. Complete the ACD-A/Heparin label with time and date of preparation and date and initials of preparer and place on the bag.
      9. Complete the **ACD-A/heparin reagent mixing log**.
   B. Selecting the Procedure
      1. Touch **Select Procedure**. The procedure selection screen appears.
      2. Touch **Mononuclear Cell (MNC) Collection**
      3. Touch **Confirm**. The system loads the procedure software.
   C. Loading the Tubing Set
      1. Touch **Prepare Tubing Set**. The screen appears instructing you to prepare the set.
      2. Unpacking the lines and bags.
         a. Put the tubing set package on top of the centrifuge cover with the label upright and facing you, and remove the cover from the package.
         b. Take out the product bags and the vent bag and put them on the right side of the centrifuge cover.
         c. Take out the coiled inlet line (red clamps) and remove the paper tape from the coil. Hang the inlet connection on the left end of the IV pole. Repeat this step with the return line (blue clamps).
         d. Take out the AC line (orange spike) and the saline line (green spike) and hang the lines over the left side of the system.
         e. Take out the cassette and the channel, and put them on top of the centrifuge cover.
         f. Discard the empty tubing set package.
      3. Loading the cassette into the cassette tray.
         a. Put the bottom of the cassette in the bottom edge of the tray.
         b. Ensure that there is nothing lodged behind the cassette of the tray that could interfere with the loading.
         c. Press the top corners of the cassette to snap the cassette into the tray. Hang the bags from left to right on the IV pole in this order: vent bag, plasma bag, collection bag.
      4. Loading the channel in the centrifuge.
         a. Open the centrifuge door.
         b. Locate the pin on the filler latch. Raise the latch by pushing the pin toward the center of the filler while pulling up the latch.
         c. Turn the centrifuge so that the loading port faces you.
         d. Fold the channel in half. Tuck the chamber inside the folded channel.
         e. Push the channel up through the loading port and the opening in the center of the filler. Lower the filler latch and lock it in place.
         f. Position the lower collar in the filler latch so that the pink line is not obstructed by the other lines.