For automated cell counting of “fresh” HCT products (i.e., samples of HPC, Apheresis or HPC, Cord Blood collected and transported at room temperature and analyzed within 24 to 48 hours of receipt), there is excellent between-laboratory agreement of TNC measurements based on results of external proficiency testing programs such as the Stem Cell Processing (SCP) and Cord Blood Testing (CBT) programs offered by the College of American Pathologists (CAP). Results from these surveys typically show CVs in the 5% to 10% range for total WBC and TNC measurements in HPC, Apheresis and HPC, Cord Blood, respectively. It is important to note that although automated hematology analyzers are also used to measure TNC in HPC, Marrow, aspirated marrow often contains numerous fat globules that interfere with optical and impedance-based cell counting.
At the present time, for this and other reasons related to availability, there are no external proficiency testing programs approved by the Centers for Medicare and Medicaid Services that routinely evaluate HPC, Marrow products. Thus, for HPC, Marrow, significant challenges remain in obtaining standardized, accurate TNC measurements that can be readily compared between laboratories. Keever-Taylor and Creer explain how to eliminate the interference of marrow lipid droplets by processing to remove the lipid. Sakamoto et al, Shibata et al, and Mori et al provide further discussion on eliminating the interference by "reconfiguration"
of the instrument and reagent combination).

**DMSO Cryopreservation and Cell Recovery**

Although TNC measurements are relatively straightforward in pre-freeze HCT products, there are several reasons to be cautious in extrapolating this experience to postthaw TNC measurements. The most important consideration to keep in mind relates to the cellular heterogeneity of HCT products and the differential effects of DMSO cryopreservation and cooling rate on viability of different cell populations. As noted above, red cells undergo lysis after the freeze-thaw cycle. Thus, NRBCs that are included in the TNC measurement before freezing exist only as “bare nuclei” after thawing and, therefore, are not generally counted as nucleated cells in a postthaw specimen by most automated hematology analyzers. (See also Fig 3-2 for illustration of calculation of expected postthaw TNC recovery in a product with 6% NRBC (ie, 6 NRBC/100 WBC.)

Granulocytes are also notoriously difficult to preserve by DMSO cryopreservation techniques and are particularly sensitive to loss of cell viability during thawing. Although the technology employed by most automated hematology analyzers results in the inclusion of both viable and nonviable cells in the reported cell count, the effects of the freeze-thaw cycle on granulocytes makes them sensitive to mechanical disruption and cell loss due to osmotic lysis, which would be reflected by a decrease in TNC recovery. Even if granulocytes do not undergo osmotic lysis, most automated hematology analyzers employ detergents in the reagents added to cells before cell counting in order to modify cell size and promote staining of cell contents to allow different cell populations to be readily distinguished and counted. Thus, increased susceptibility to detergent-mediated cell disruption after thawing could also contribute to substantial reduction of TNC recovery in granulocyte-rich HCT products. This is likely to be a particular concern for HPC, Cord Blood and for HPC, Marrow, where granulocytes generally account for 40% to 60% of TNC. However, it is also a significant concern for HPC, Apheresis where granulocytes still make up a significant fraction of the TNC content.

Mononuclear cells (lymphocytes, monocytes, and HPCs) are more optimally preserved by DMSO cryopreservation using cooling rates in the –1 to –8 °C/minute range which, as described previously, were specifically selected to provide optimal preservation of HPC viability. Accordingly, the effects of freeze-thaw are expected to have significantly less impact on the recovery of the mononuclear cell fraction compared to granulocytes and NRBCs in HCT products.

**DMSO and Washing**

Another major factor influencing TNC recovery after thawing is
product manipulation. Concern over DMSO toxicity to the recipient and to the potential adverse effects of DMSO on postthaw cell viability has led many centers to routinely adopt procedures to “wash” products to remove DMSO. One of the most common washing procedures\textsuperscript{12-13} employs centrifugation and multiple transfer steps and results in TNC loss that approaches 20%.\textsuperscript{14} This magnitude of cell loss has minimal impact on transplant outcome for most recipients of HPC, Apheresis and HPC, Marrow, but is a major concern for HPC, Cord Blood, where time to engraftment and overall survival show a strong relationship to administered cell dose.\textsuperscript{15-16} Alternative approaches to thawing and infusing HPC, Cord Blood have been developed that do not require removal of DMSO. For these approaches, TNC recovery is 10% to 20% higher than that of products washed to remove DMSO.\textsuperscript{17-19} Accordingly, washing to remove DMSO should probably be performed only for small neonates where concern over DMSO toxicity arises (more than 1 mL DMSO infused /kg body weight) and in situations of major ABO incompatibility. Because TNC recovery is strongly influenced by the degree of postthaw manipulation, it is very important to take this into consideration when using TNC recovery to assess the impact of the cryopreservation and freeze-thaw process on cell survival.

**DMSO and Cellular Characteristics**

A third factor affecting postthaw TNC measurements relates to the potential effects of DMSO on the cellular characteristics measured by automated hematology analyzers. These characteristics include cell size, granularity, light-scattering properties, and dye-binding characteristics. Different analysers use a variety of cytochemical and enzyme histochemical techniques to characterize and count WBC and NRBC populations. In the author’s experience using the Sysmex XE series (Kobe, Japan) automated hematology analyzers, there is minimal impact on TNC measurements in undiluted HPC, Apheresis and HPC, Cord Blood samples suspended in media containing 10% DMSO for up to several hours before freezing and after thawing.\textsuperscript{10,20} The most likely explanation for this is the extensive dilution of the cell sample with the reagents used for cell counting (typically 1 part cell suspension to 200 or more parts cell counting reagent) and the reversibility of the effects of DMSO on the cell characteristics being measured after dilution. Because other automated hematology analyzers employ similar extensive sample dilution and cell counting technology, DMSO effects on cell counting may also be insignificant but this has not been well studied.